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MODES OF SELECTION*

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INTRODUCTION

In order to get to the topics which I wish to discuss, it will be necessary to begin with a rather dogmatic statement of my general position with respect to evolution.

Adaptation rather than mere change seems to me to be the central problem. The only mechanism for evolutionary adaptation that has held up under investigation is natural selection.

Selection can not, of course, operate without raw material in the form of undirected genetic variability. This may include both inconspicuous and conspicuous gene mutations, chromosome aberrations of all sorts, genetic material from genes to genome introduced by hybridization, genotypes as wholes produced by recombination, and differences among populations from local ones within species to competing species and higher categories. All of these are raw materials which require natural selection for assemblage into patterns that constitute significant evolutionary change.

The topic which I discuss is a comparison of the modes of selection at various levels of organization.

SELECTION AMONG GENES AND CELLS

At the lowest level is selection among genes in mere capacity to persist and in mechanism of exact duplication. It seems probable, as suggested by Darwin (cf. Hardin 1950) and developed by Haldane (1933) and Oparin (1938), that life originated in an organic soup that could only have accumulated in the prior absence of life and that was one that provided all necessary metabolites. It is hardly probable that the chemical basis for gene persistence and duplication appeared full fledged. We may conclude with Blum (1951) that evolution took the form at first of decreasing mutability. Along with this may have occurred the evolution of stable genic association in cells, and mechanisms of exact duplication of the entire system by mitosis.

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*Address of the President, Society for the Study of Evolution, delivered at the annual meeting, Austin, Texas, April 10, 1955.

With the attainment of a sufficient degree of stability in these respects, but increasing depletion of natural resources, selection for capacity to take necessary metabolites from other organisms, or to synthesize them from precursors would take precedence over selection for further stability (Horowitz, 1945). Decreasing mutability would, however, continue to be important whenever evolutionary increase in the length of the life cycle occurs.

SELECTION UNDER EXCLUSIVE UNIPARENTAL REPRODUCTION

Exclusive uniparental reproduction leads to the formation of clones of constant genotype, except for rare mutations. Selection between clones is an extremely effective process since the selective value of the genotype as a whole is the basis. Even very slight differences in rate of reproduction, in a limited environment, leads rapidly to extinction of all but the most rapidly multiplying clone (Haldane 1932). Thereafter, however, the material for evolution is restricted to the mutations that arise from time to time. Of two mutations that are present at the same time in a competing population, only one persists. Recombination requires mutation in tandem in the same line.

MASS SELECTION

Biparental reproduction gives the great advantage that the evolution of the entire population, rather than a single lineage, is promoted by selection. Mutations that arise in different individuals may later be combined. A store of variability is maintained in various ways. Evolution may occur simultaneously in all characters.

The ways in which the frequencies of favorable mutations increase in a great variety of circumstances were presented by Haldane in a series of early papers summarized in 1932.

THE PLEIOTROPIC THRESHOLD

An important question is whether there is a threshold in the selective advantage of a gene over its alleles which must be exceeded for effective operation of natural selection. As far as over-all selective advantage is concerned, it is clear enough from Haldane's analysis that there is no such threshold, although selective difference of, for example, 10^{-6}, would require a great many generations to produce an appreciable change in gene frequency.

Selection of a particular character is, however, a very different matter. This is because of the prevalence of pleiotropy, using the term in the broad sense to include not only primary pleiotropy (effects of the gene itself on more than one process) but also secondary pleiotropy (effects on different characters that branch out from a single primary effect). The epigenetic nature of development implies secondary pleiotropy as an almost universal principle. Inspection of such lists of mutant effects as given by Bridges and Brehme (1944) for *Drosophila melanogaster* indicates that this is in fact the case, even at the level of gross effects on viability, fecundity, color and morphology. It is probable that investigation focused on this question would reveal pleiotropic differences for all distinguishable alleles.

If we analyze the total selective value (W) of a gene into terms (W_1, W_2, W_3, etc.) that relate to characters that are known to evolve largely independently, total selective value may be considered as the product of the components, or in terms of selective advantage ($S = W - 1$), the total is approximately the sum of the contributions, assuming that all S's are small. If $S_1 = -.0001$ and $S_2 = +.01$, it is obvious that the gene will tend toward fixation because of the favorable effect on the second character, in spite of its unfavorable effect on the first. If $S_1 = -.0001$ and $S_2 = -.01$ it will tend toward extinction, but not to an important extent because of its unfavorable effect on the first character. In the class of genes that have the selective disadvantage 10^{-4} for a particular character, this effect decides the issue only for those for which the coefficient for residual effects is less than 10^{-4}.

These considerations probably have an important bearing on the complete loss of useless characters or their persistence as vestiges (Wright 1929). No doubt selection against such characters as encumbrances is the most important factor in their degeneration up to a certain point. Beyond this, selection based on pleiotropic effects may be expected to take over. The genes on which the useless character depends may be expected to be involved in other characters. When the direct value to the organism of further degeneration becomes small enough, persistence or loss depends largely on the selection coefficients relative to these. If the organism is conservative in these respects, the favorable selection coefficients may maintain the useless character as a vestige for a very long time, in spite of a slight negative component for the effect on the useless character by itself. If, on the other hand, there is considerable reorganization of these other characters, the selection coefficients favoring the replacing alleles will tend to bring about relatively rapid complete loss of the useless character as a byproduct.

The pleiotropic threshold also has an important bearing on theories of the evolution of dominance (Wright 1929, 1934), a matter on which my position has often been misrepresented. (e.g. in Fisher 1949 and earlier)

MULTIPLE PEAKS IN SELECTIVE VALUE

The most serious difficulty with mass selection as the primary guiding principle in evolution is that it does not apply to the genotype as a whole but only to the net effects of the individual genes taking account of all of the genotypes in which they enter. This is because genotypes, formed in one generation, are broken up in the next, by the reduction division, and the random union of gametes.

This limitation would not be serious if each gene replacement always contributed to selective value in the same sense in all combinations, since in this case there would always be just one goal (in terms of the genes currently present) toward which the species would move under mass selection from any initial composition. The same result would occur even if there were no contribution in some combinations with genes at other loci or even if there were reversal of effect in isolated cases.

There may, however, be two or more peaks, even at a single locus, such that mass selection may lead to a lower peak from certain initial states and hold the population there the more firmly, the more severe the selection. The simplest case is, of course, that of two alleles with a heterozygote inferior to both homozygotes. This is probably not very common. The case of semi-sterility due to reciprocal translocation can, however, be treated formally as of this sort in plants in which the aneuploid gametophytes are eliminated. The case in animals is more complicated but somewhat similar and will be discussed later.

A situation that is probably of very considerable evolutionary importance may occur in the common case in which superiority of the heterozygote A_1A_2 of two alleles maintains a population in a heterallelic state. A mutation A_3 may occur which combines the favorable effects of A_1 and A_2, and if fixed would produce a population superior to the previous heterallelic one (Haldane 1937). If the heterozygotes A_3A_1, A_3A_2 (assumed equal in selective value) have more than half the selective advantage of the original heterozygote A_1A_2 over the two homozygotes A_1A_1 and A_2A_2 (also assumed equal), all three alleles are retained (or A_3 is fixed), in a population with a single peak. If however A_3A_1 and A_3A_2 have less than half the advantage of A_1A_2 over A_1A_1 and A_2A_2 there are two peaks. Mass selection may keep the population essentially heterallelic for A_1 and A_2 only, (in spite of mutations to A_3), even though a population homallelic in A_3 would be superior (based on formula in Wright 1949a).

Similar situations can occur in combinations of two pairs of alleles. It could happen, for example, in the selection of guinea pigs for a rosette on the forehead. Assuming a certain common genetic background (*mm*), genotype *rr stst* has no forehead rosette, *R-stst* has a rather well developed one, *rr St-* has a very well developed one, while the combination *R-St* has only a slight irregularity in this region (Wright 1950).

There are other cases of this sort in the guinea pig, but it must be admitted that a list of such special cases does not indicate that factor interaction would often interfere seriously with progress under mass selection.

If, however, we focus on selective value of the genotype as a whole, as we must in dealing with natural selection, in contrast with artificial selection for special characters, it may be seen that a multiplicity of peak genotypes may be expected to be the rule.

In any species that has been exposed for a long time to similar conditions, the optimum grade of most quantitatively varying characters is to be expected near the mean. Assuming determination of the grade by the cumulative effects of multiple factors as in the conventional theory, the same gene replacement that is favorable in genetic backgrounds sufficiently below the optimum is unfavorable in ones above. Grades, near the optimum, are given by a number of different homozygous genotypes, differing by two or more steps and thus at independent peaks in the surface of selective values. Mass selection tends to fix one of these, according to the initial gene frequencies, or at most, to maintain one locus in a heterallelic state (Wright 1935).

It may seem of little evolutionary significance which peak is attained since all have about the same selective value by hypothesis, but this is only according to the effects on this particular quantitative character. Taking pleiotropic effects into account, there may still be multiple peaks, but with widely different selective values.

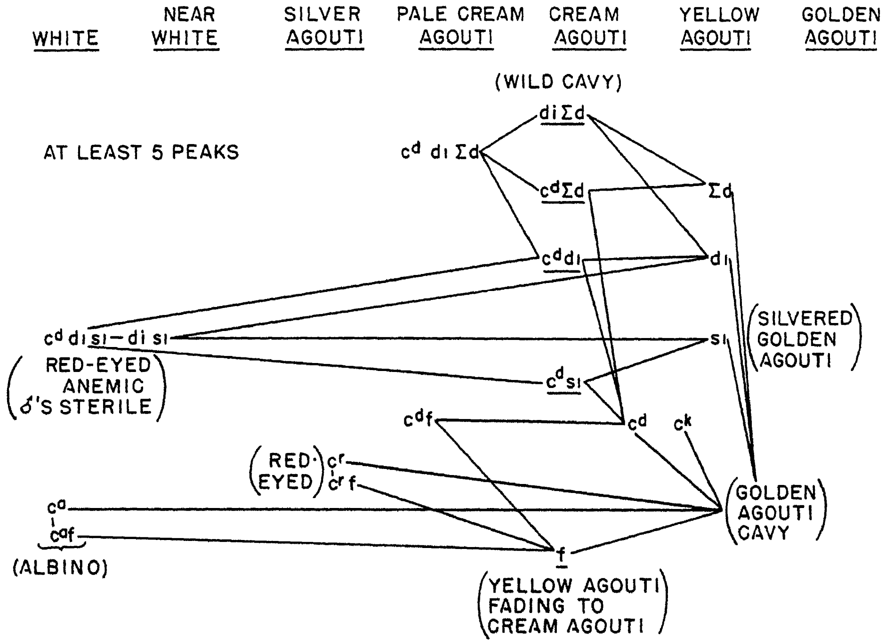
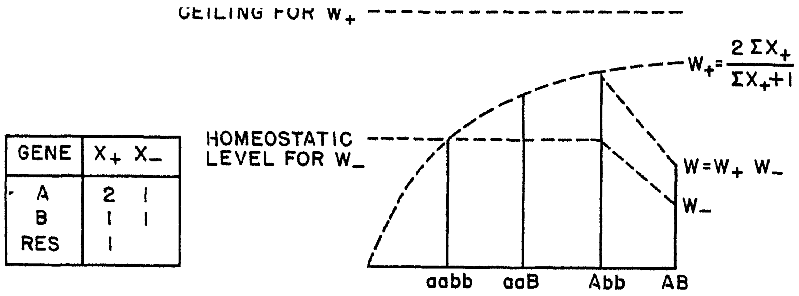


FIGURE 1. Certain genotypes of the guinea pig and wild Peruvian Cavy *(Cavia cutleri)* according to difference from the golden agouti variety. The protectively colored cream agouti color of the wild cavy is definitely not due to an albino allele (c^dc^d or c^kc^k), which gives cream agouti in association with minor dilution factors (such as *si* and *di*). It differs from the golden agouti by multiple minor factors (Σd) possibly including the factor *di* of the guinea pig. There are at least 5 adaptive peaks due to this intermediate optimum.

Figure 1 is a simplified illustration of such a case. The color of the Peruvian Cavy, *Cavia cutleri*, the probable wild ancestor of the guinea pig, is like the guinea pig color known as cream agouti (Castle in Castle and Wright, 1915). It is well adapted for protective concealment. It is intermediate between silver agouti (or the even more extreme white) and the intense golden agouti color. Cream agouti guinea pigs, resembling more or less closely the color of the wild cavy, can be produced in many ways, but the results of crosses indicate that none of these is as close genetically as is the golden agouti. There are probably at least five homozygous cream agouti genotypes each at two or more removes from each of the others

(Wright, 1949b). All are thus at different adaptive peaks in the surface of mean selective values with respect to protective coloration. There are, however, differences in the intensity of the sepia in the hair, even though the subterminal cream band is the same, and differences in the amount of fading of later pelages, that make it certain that not all peaks would have the same adaptive value.

Even if the optimum grade of a character is beyond the current range, pleiotropy can easily give rise to multiple peak genotypes. A number of model cases are illustrated in figures 2 to 8. The simplest is figure 2. Dominant genes *A* and B are supposed to contribute cumulatively but unequally to the grade of a favorable character, and also cumulatively, and in this case equally, to an unfavorable one. Residual factors (Res.) contribute only to the former and are easily brought to near-fixation by selection. It is assumed that there is damping of the favorable contributions as the totals


CEILING FOR W_+
$W_+ = \frac{2\Sigma X_+}{\Sigma X_+ + 1}$
HOMEOSTATIC LEVEL FOR W_-
$W = W_+ W_-$
W_-
aabb
aaB
Abb
AB

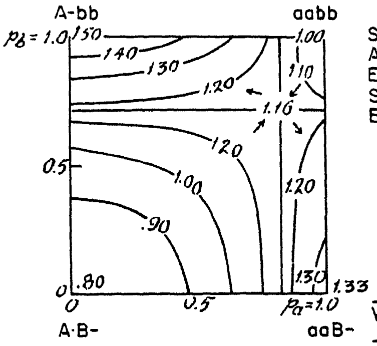
GENE	X_+	X_-
A	2	1
B	1	1
RES	1	

MEAN SELECTIVE VALUE IN A SIMPLE CASE OF PLEIOTROPY

FIGURE 2. Genes *A*, *B* (and residual heredity Res.) contribute favorably (X_+) to one character, but *A* and *B* tend to contribute unfavorably (X_-) to another. The component of selective value due to the former (W_+) is subject to damping as a ceiling of possible favorable effect is approached. The component (W_-), due to the latter, is under homeostatic control with respect to the effects of *A* and *B* separately, but this breaks down in the combination *AB*. Populations homallelic in *AAbb* and *aaBB* are at distinct peaks in net selective value (*W*).

increase, at least in selective value, in accordance with the almost universal principle of diminishing returns from increasing application of any one kind of factor. Another sort of factor becomes the limiting one. This is analogous to the theory of dominance and of the extreme lability of intermediate heterozygotes (Wright 1929) but is here applied to combinations of loci (Wright 1934). Rasmussen (1933) has discussed such damping in connection with crop yields.

The tendency toward unfavorable effects by *A*- or *B*- each by itself, is assumed to be prevented by homeostasis built up in the past against this sort of effect, but it is assumed that the combination *A*-*B*- deviates more than can be taken care of in this way. The result with the selective values chosen is that *aaB*- and *A*-*bb* are peak genotypes at different levels, while *A*-*B*- is even less well adapted than *aabb*. Figure 3 shows the surface of mean selective values, $\overline{W}$, by means of contours.



MEAN SELECTIVE VALUE IN SIMPLE CASE OF PLEIOTROPY ASSUMING DAMPING OF FAVORABLE EFFECT AND HOMEOSTASIS FOR SINGLE DOSE OF UNFAVORABLE EFFECT.

GENE	X_+	X_-
A	2	1
B	1	1
RES.	1	

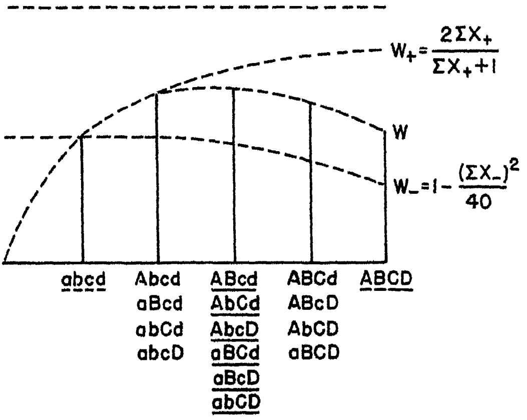
$$\bar{W} = 1.00\, p_a^2 p_b^2 + 1.33\, p_a^2 (1-p_b^2) + 1.50\, (1-p_a^2)\, p_b^2 + .80\, (1-p_a^2)(1-p_b^2)$$

FIGURE 3. The contours on the surface of net selective value (W) in the case shown in figure 1.

It is evident that mass selection might lead to either one of these peaks, depending on the initial composition of the population, and, if the lower one, it would prevent attainment of the higher one. Artificial selection for the favorable character would, on the other hand, tend toward fixation of genotype *AABB* with disastrous effects with respect to the unselected character. It is notorious that strong artificial selection usually has this sort of consequence (cf. Mather and Harrison 1949, Lerner and Dempster, 1951, Robertson and Reeve 1952a and many others).

Figure 4 represents a case in which exclusively favorable residual heredity is supplemented by four dominant factors with equal favorable effects, and also with equal unfavorable effects on a second character. The law of diminishing returns is again assumed to apply to the favorable character and homeostasis to the unfavorable one. The chosen values result in a situation similar to that with an intermediate optimum. With four pertinent

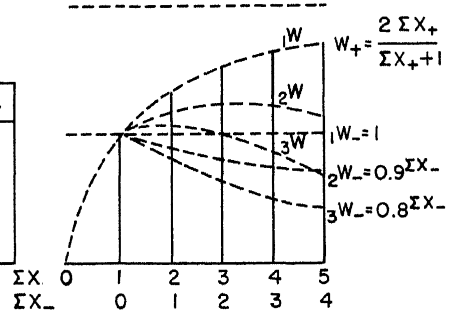
GENE	X_+	X_-
A	1	1
B	1	1
C	1	1
D	1	1
RES.	1	



2 PITS
6 PEAKS

FIGURE 4. Similar to figure 1 except that four dominant factors contribute favorably to one character, subject to damping, and unfavorably to a second, subject to decreasing homeostatic control, in both cases as the cumulative primary effects increase.

GENE	X_+	$_AX_-$	$_BX_-$	$_CX_-$	$_DX_-$
A	1	1			
B	1		1		
C	1			1	
D	1				1
RES.	1				



	abcd	Abcd etc.	ABcd etc.	ABCd etc.	ABCD		
$_1W$	1.00	1.33	1.50	1.60	1.67	1 PIT	1 PEAK
$_2W$	1.00	1.20	1.22	1.17	1.09	2 PITS	6 PEAKS
$_3W$	1.00	1.07	0.96	0.82	0.68	2 PITS	4 PEAKS

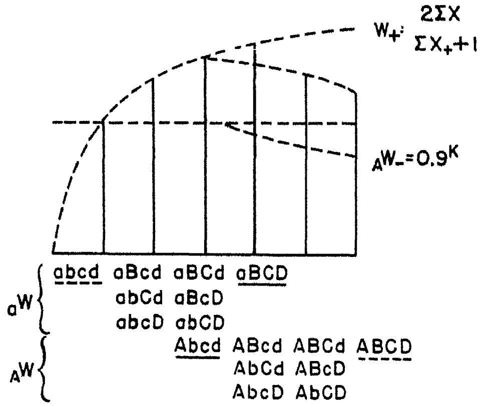
MULTIPLE FAVORABLE FACTORS WITH INDEPENDENT UNFAVORABLE EFFECTS

FIGURE 5. Similar to figure 3 but unfavorable effects of the four factors are on distinct characters, subject to the independent homeostatic control.

loci there are adaptive pits at each extreme (*aabbccdd* and *ABCD*) and six adaptive peaks, corresponding to the combinations with any two dominant factors. The peaks are all at the same level in this case but this is because of the complete identity assumed for the effects of the four loci and would not be the case in general.

It is of interest to compare with this a situation which is the same except that the unfavorable tendencies of the four genes are each for a different character (Figure 5). It is evident that there is considerably less likelihood of multiple peaks since each of the unfavorable tendencies may be

GENE	X_+	$_AX_-$	$_BX_-$	$_CX_-$
A	2	1	1	1
B	1	1		
C	1		1	
D	1			1
RES.	1			



K = NO. OF CHARACTERS
BELOW HOMEOSTATIC
LEVEL.

2 PITS
2 PEAKS

FIGURE 6. A major factor (*A*), three minor factors (*B,C,D*) and residual heredity (Res.) contribute favorably to one character subject to damping. Factor *A* has three independent unfavorable pleiotropic effects. Factors *B*, *C* and *D* have unfavorable pleiotropic effects each on one of those affected by *A*. Net selective value of populations allowing for damping of favorable effects and homeostatic control of unfavorable ones shows two widely separated peaks, *Abcd* and *aBCD*.

subject to homeostatic buffering, independently of the others. There is no necessity however that the unfavorable effects should be wholly prevented. Various numbers of peak genotypes may be present depending on the amounts of the various effects.

It may be added that the existence of multiple peaks is also not dependent on damping of the favorable effects of the genes, although more likely with such damping than without.

In actual cases it would be expected that some of the unfavorable pleiotropic effects would be on the same character (from the standpoint of homeostatic control) and others on different ones. Figure 6 is a model of such a case. With the chosen selective values there are two peaks, a lower one, *A- bb cc dd*, and a higher one, *aa B-C-D-*, separated by a wide and deep valley (four steps). In general, there would seem to be more likelihood of reaching a higher grade of an adaptive character by a combination of multiple minor factors with only minor and independent deleterious tendencies, if any, than by a major factor, more likely to have multiple serious deleterious tendencies which would tend to supplement those of other, otherwise favorable, factors.

TABLE 1

MACROMUTATION A INCREASES SELECTIVE VALUE 50% IN ONE RESPECT, REDUCES SELECTIVE VALUE 20% IN EACH OF 3 RESPECTS.

EACH OF GENES *B*, *C* AND *D*, IN PRESENCE OF *A*, RAISES SELECTIVE VALUE IN A DIFFERENT ONE OF ABOVE RESPECTS TO .90. EACH CAUSES 1% DECREASE INDEPENDENTLY OF *A*.

Genotype	W	Genotype	W
abcd	1.00	*Abcd*	.77
aBcd etc.	.99	*ABcd* etc.	.85
aBCd etc.	.98	*ABCd* etc.	.95
aBCD	.97	*ABCD*	1.06

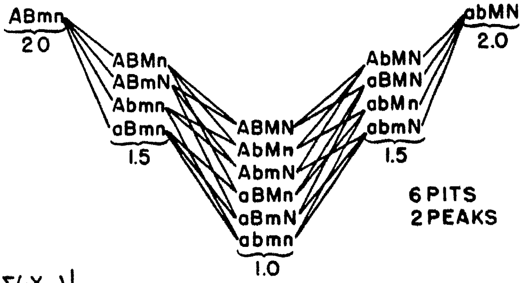
A can become abundant only where *BCD* predominates.

The most probable way by which a major factor of the above sort might be utilized is by joint fixation of relatively specific modifiers of its unfavorable effects in spite of slight disadvantageous effects of the latter. (Table 1). This is an example of what Dobzhansky and Wallace (1950) call coadaptation. (cf. also Wallace, 1953.)

INCOMPATIBLE ADAPTATIONS

Another important sort of case in which more than one peak genotype is to be expected is that in which there are more or less incompatible ways by which the species may meet the challenge of the same environment. The case of complete incompatibility of two such adaptations is illustrated in figure 7. The situation represented is almost the opposite of the case in figure 4 in that there are two peaks at opposite extremes and six intermediate pits. If incompatibility is not complete, however, as illustrated in figure 8, there may be three peaks, the two modes of adaptation in pure

GENE	${}_1X_+$	${}_2X_-$
A	1	1
B	1	1
GENE	${}_1X_-$	${}_2X_+$
M	1	1
N	1	1



$W = 1 + 1/2\,|\Sigma({}_1X_+) - \Sigma({}_2X_+)|$

100% RECIPROCAL INTERFERENCE BETWEEN CHARACTERS

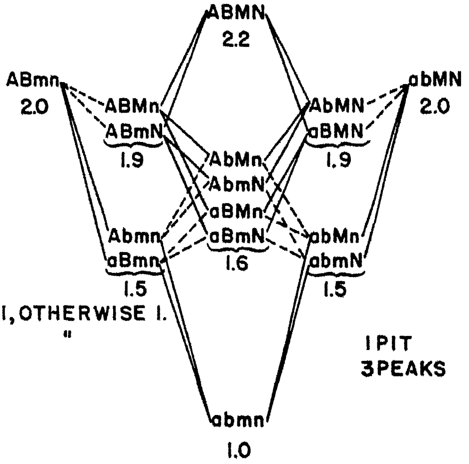
FIGURE 7. Genes *A* and *B* contribute toward one adaptation, *M* and *N* toward a completely incompatible alternative. There are two widely separated peaks in selective value (*ABmn*; *abMN*).

form *ABmmnn*, *aabbMN* and a combination (*ABMN*) that may have a higher selective value but be inaccessible from the multiple recessive as the starting point.

Recent selection experiments of King indicate that resistance of Drosophila to DDT may be of this sort. Marked success in two different lines was maintained in F_1 but largely lost in F_2.

A situation similar to the last model occurs with respect to adaptation for slightly different ecological niches throughout the range of the species (different adaptive subzones of Simpson (1944)), on measuring mean selective value by weighting the selective values in the different niches by their prevalence.

GENE	${}_1X_+$	${}_2X_-$
A	1	0.4
B	1	0.4
GENE	${}_1X_-$	${}_2X_+$
M	0.4	1
N	0.4	1



${}_1W = 1 + \Sigma({}_1X_+) - \Sigma({}_1X_-)$ if > 1, OTHERWISE 1.
${}_2W = 1 + \Sigma({}_2X_+) - \Sigma({}_2X_-)$ " "
$W = 1/2\,({}_1W + {}_2W)$

40% RECIPROCAL INTERFERENCE BETWEEN CHARACTERS

FIGURE 8. Similar to figure 7 but with only partial incompatibility. The highest peak of selective value (*ABMN*) is widely separated from two other peaks, *ABmn* and *abMN*.

The above models refer only to single favorable characters in each case. Actually the surface of mean selective values depends on all loci, considered simultaneously.

If the eight favorable characters that have been considered are assumed to evolve independently of each other, the net selective values are the products of the values indicated for each case. The total number of peaks is thus 8640 ($= 2\times5\times2\times6\times6\times2\times2\times3$) (taking the largest number in the fourth case). Twenty independent characters averaging 4 peaks each would give 10^{12} peaks. Actually, one would expect many more pleiotropic effects and extensive interlocking of characters.

One would also expect more heterallelism at peaks than indicated above in view of the accumulating evidence that overdominance is common. (East 1936, Crow 1948, Reeve and Robertson 1952, Dobzhansky and Wallace, 1953, Lerner 1953).

EFFECTS OF CHANGING ENVIRONMENT

It has been assumed in the preceding discussion that the conditions of selection are constant, apart from heterogeneity within the range, in the last case. In nature, the conditions are continually changing, sometimes only very slowly but sometimes rapidly. This means a continual shifting of the goal toward which mass selection is directed and in general a continual tendency toward loss of adaptation. It also means, however, that the species is kept continually on the move on the changing surface of selective values, instead of being bound by the mass selection to a single peak. The species is on a sort of treadmill but not a stationary one. It tends to work its way gradually toward the permanently higher regions in the surface. "Here we undoubtedly have," as noted in an earlier paper (Wright 1932), "an important evolutionary process and one that has been generally recognized."

It is fairly obvious, however, that even in this case, the process of continual readaptation would be more effective if it could be based on the adaptiveness of genotypes or systems of these, than merely on the momentary net effects of the separate genes. It is thus a question of primary importance in the theory both of evolution in a steady and in a changing environment, whether there is any way in which selection can be of the more effective sort referred to above.

ALTERNATION OF UNI- AND BI-PARENTAL REPRODUCTION

One possibility is a combination of prevailing uniparental reproduction with occasional crossbreeding. As already noted selection among clones is by the genotype as a whole and is an extremely effective process. If crossing occurs while there are still widely different genotypes on hand in the competing populations, recombination will provide an extensive new array of clones, all probably novel, but based on the successful ones of the preceding selection cycle. The field of variability is comparable to that under random mating but the binding of the population to a single adaptive peak is avoided. There is the effective selection by genotype found in a heterogeneous array of clones but not the destruction of the store of variability and resulting stagnation that occurs under exclusive uniparental reproduction. The combination of the two systems has synergic effects (Wright 1931a).

This is a system that occurs in many organisms: viruses, bacteria, protozoa, trematodes, rotifers, cladocera, aphids, and many plants. It does not, however, predominate in the groups that have evolved the most complex adaptations: most higher plants, most arthopods, cephalapods and especially the vertebrates.

It is a system of violent alternations. The selection of favorable genotypes is followed by the breaking down of these by crossing, except in those respects in which all successful clones are alike, and in these respects no new field of variability is re-established by crossing. It is a system that is probably more significant as a means of insuring the appearance of adaptive genotypes, capable of taking advantage of any temporary bonanza, whatever the special conditions, than of progressive evolution.

INTERDEME SELECTION

A more effective way by which selection may be based on genotypes as wholes or (better) systems of such genotypes is by what I have called in early papers intergroup selection (Wright 1931a, b) but for which interdeme selection is perhaps a better term. The members of any reasonably large species do not actually interbreed at random. Most species occupy such extensive ranges in relation to the dispersal of individuals that random mating is impossible. Moreover, it is unusual to find the range occupied with anything like uniform density. The individuals are found in largely isolated communities where the local conditions are favorable. Dr. George Carter (1951) has stated "without doubt, the organization into communal populations is a general and fundamental fact of natural history. For this population, the term '*deme*' has been proposed" (Gilmour and Gregor 1939).

The demes are not completely isolated. Selection, with the deme as its unit, takes the form of more rapid growth of population of the better adapted ones and their more extensive dispersion into the territories of the less adapted with consequent grading up, or even replacement, of the latter.

Like any other mode of selection, interdeme selection can operate only if there is a source of variation among its objects that is independent of the course that ultimately emerges. In this case, the significant variability is differentiation of the demes. This variability can best be described in terms of the array of gene frequencies at all heterallelic loci. Selection is here according to *sets* of gene frequencies, the mean selective values of which depend on the communal adaptiveness, or coadaptation, of the whole set of genotypes, rather than on the net effects of genes or even of genotypes. Robertson and Reeve (1952), Dobzhansky and Wallace (1953), Lerner (1953), and others have recently stressed the importance of heterallelic systems in adaptation and in adaptive evolution.

The most obvious cause of differentiation of demes is, no doubt, adaptation to different local conditions by local mass selection. This process may sometimes lead to genetic complexes in one locality that have general value, but ordinarily this is not the case. The most important result is to give the species wide adaptability and a large store of variability. If differentiation is great enough there is splitting of the species into recognizable subspecies and even species. Splitting of species is a most important evolutionary phenomenon, but we are here considering transformation as a single species.

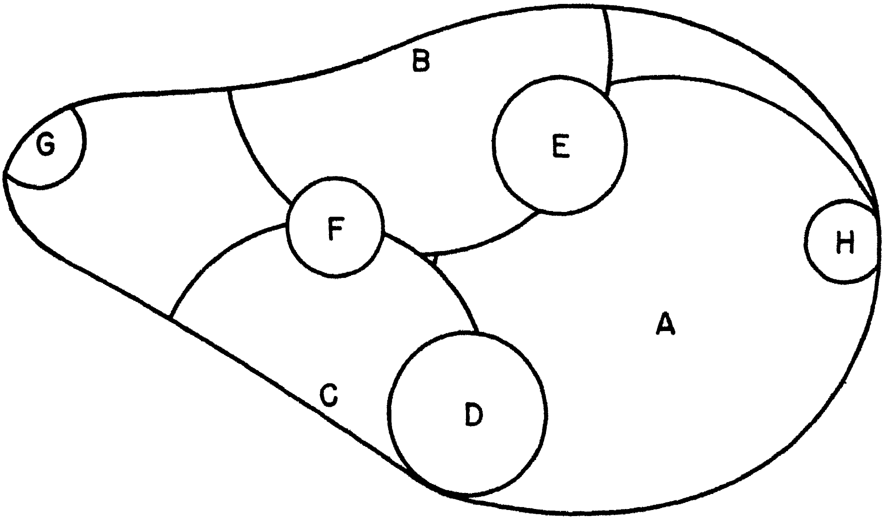


FIGURE 9. Interdeme selection within the range of a species, under uniform conditions. Relatively adaptive demes A, B, C, G arise from time to time from joint action of random processes and local mass selection. These expand, suffering modification in the process. New, more highly adaptive demes arise, especially in regions of contact (D,E,F) of the zones of influence of previous adaptive demes.

There are several ways in which random differentiation of demes may occur under the same prevailing conditions. Comparisons can be made by finding the distribution of gene frequencies at loci when specified rates of recurrent mutation, specified amounts of immigration and mean gene frequency of immigrants, specified mass selection coefficients (all of which tend to shift gene frequency toward a certain equilibrium point), are in statistical balance with the scattering due to random processes. Among the latter are the cumulative effects of accidents of sampling in small populations (Wright 1931a), fluctuations in the position of an intermediate optimum (Wright 1935) and fluctuations in the coefficients for the various directed processes referred to above. The nature in specific cases has been shown in a paper in 1948 on the basis of a general formula, presented earlier (Wright 1938).

The term random drift has often been applied solely to effects of accidents of sampling but should properly be applied to all random processes (in contrast with the steady drift from the directed processes) since as far as the theory of evolution by interdeme selection is concerned, the kind of random process that provides its raw material is largely irrelevant.

SOME MISUNDERSTANDINGS

There has been considerable misunderstanding of the role which I attribute to random drift. People like explanations in terms of single factors. Thus they like to think of characters as either wholly hereditary or wholly environmental. They tend to support one or another factor (mutation, hybridization, selection, etc.) as the essential factor of evolution and to classify theories of others similarly. If a certain change is shown to require a macromutation it is argued that the gradual accumulation of small differences is ruled out as a valid evolutionary phenomenon, or vice versa. If evidence is found for a selective difference between particular populations, this is held to prove that random processes have played no role in this, and again vice versa.

I have sometimes been labelled as an exponent of random drift as the only significant evolutionary phenomenon. When I deny this, a quotation from one of my early papers, (Wright 1931b), stated to apply to "non-adaptive radiation" is offered in evidence (Fisher and Ford 1950, Wright 1951). "In short, this seems from statistical considerations to be the only mechanism which offers an adequate basis for a continuous and progressive evolutionary process." This would seem to make my position in 1931 clear enough to the reader unless he is wary enough to go back and see what I actually wrote. If he does so, he will find that the two sentences preceding the quoted sentence were as follows: "The process of *intergroup selection* may be very rapid as compared with mass selection of individuals among whom favorable combinations are broken up by the reduction-fertilization mechanism in the next generation after formation. With partial isolation and differentiation, accompanying expansion of the successful subgroup, the process may go on indefinitely."

If my position in 1931 is to be labelled with a single word it would obviously have to be selectionist since "selection" is the noun antecedent to "this" in the quoted sentence, but this by itself would be highly misleading without adding that the mode of selection that seemed to me most effective is one that depends on the synergic effects of most of the other suggested factors of evolution.

In this connection, there is another misunderstanding that I would like to clear up if possible. The role that I have attributed to random drift in the process of interdeme selection is not in most cases that of bringing about *fixation* of one or another allele locally. Fixation may occur, but I have always held that "the end can only be extinction for a group permanently reduced below a certain size of population, in relation to other evolutionary factors" to quote from the same 1931 paper as above. What has been stressed has been *variation* of the gene frequencies as the condition at loci most favorable for the process, recognizing that the amount of variation that is in a steady state will differ greatly at different loci in the same population in accordance especially with the severity of the local mass selection acting on each.

The reason is that fixation completely removes the locus from control by mass selection and leads to the extreme inbreeding effect referred to. Mere variation of gene frequency on the other hand implies a joint process in which mass selection prevents seriously deleterious inbreeding effect and can at any time amplify possibilities inherent in a chance momentary pattern of the gene frequencies at multiple loci, that happens to have been arrived at, while all are varying under the random processes. The difference is that between a frozen situation and a labile one.

SYNERGIC EFFECTS OF RANDOM PROCESSES

The synergic effects of different kinds of random processes has been illustrated by the case of long time fluctuations in the position of an intermediate optimum (Wright 1935). Selective value at two different times is plotted against the grade of a conventional multifactorial character in figure 10. Suppose that there is slow fluctuation between these situations throughout a species that is subdivided into many partially isolated demes. As already noted, mass selection in each of these leads toward approximate fixation of one of the peak genotypes (or at most heterallelism at one locus). If all of the demes start from near-fixation of the same peak genotype, a small lowering of the optimum, persisting for a long time, tends to bring about loss of one of the plus factors, but a different one in different cases according to slight differences in the frequencies of these (figure 11). On return for a long time to the higher optimum, there is approach in all cases to a peak genotype with the original number of plus factors, but which set again depends on small random differences in the gene frequencies among

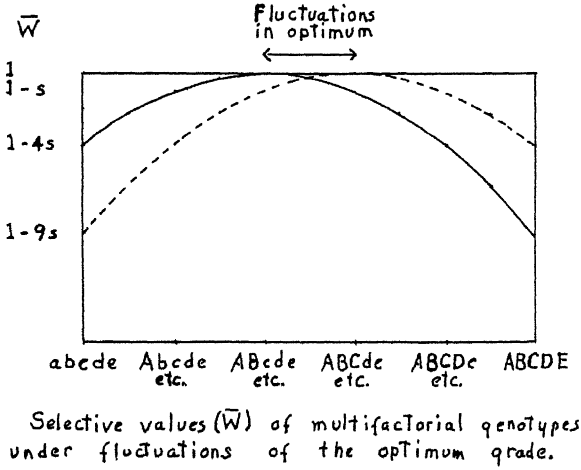
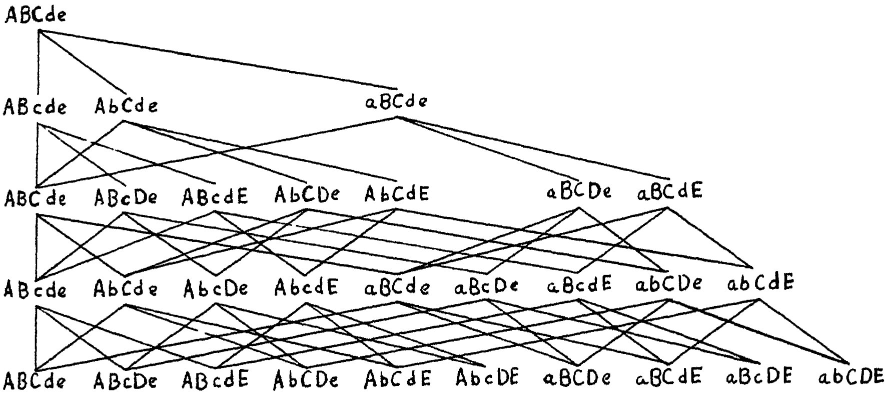


FIGURE 10. Selective values of the grades of a multifactorial character with intermediate optimum which shifts between two values.



Local differentiation from fluctuations in the optimum of a multifactorial character.

FIGURE 11. Array of possible predominant genotypes in a deme that may arise from random drift due jointly to a fluctuating optimum of a multifactorial character and accidents of sampling.

the demes. After a few such fluctuations, it is to be expected that all peak genotypes under each set of conditions will have approached fixation in some deme at some time. If there is one that has a considerable advantage because of pleiotropic effects the opportunity is given for effective interdeme selection and ultimate predominance of this genotype at least in one phase of the fluctuating conditions, and the best adapted at one remove in the other phase.

If selection proceeded to fixation in each phase, the accidents of mutation could determine which genotype would prevail in the next phase, but complete fixation under selection is an exceedingly slow process.

MACROMUTATIONS

There are probably, as Goldschmidt especially has urged (1940, 1952), important evolutionary changes in direction that rest primarily on single macromutations. There is no necessity to suppose that these become established by the abrupt origin of a new viable species by a concomitant isolating mechanism. Macromutations probably always bring about serious maladjustments and are strongly selected against, in spite of the occasional possibility of a major adaptation if the maladjustments can be smoothed out. Such mutations may be maintained at low but appreciable frequencies by recurrent mutation especially if the severity of selection is partially mitigated by low penetrance. It is exceedingly unlikely that random processes would ever bring about direct fixation. There is a reasonable chance, however, that random drift among the gene frequencies of *modifiers* may lead to a constellation in some deme at some time in which the sign of the selection coefficient of the macromutation becomes positive and it, along with its array of modifiers, becomes locally established and ultimately makes over the whole species by interdeme selection. (Wright 1951)

RECIPROCAL TRANSLOCATIONS

In cases in which there is enormous selection against a mutant heterozygote, but not against the mutant homozygote, the latter may be established by random drift if this drift also is enormous in amount. The most important case is that of a reciprocal translocation in a population with exclusive biparental reproduction. The selection against the translocation is enormous assuming the heterozygote to be semisterile as is usually the case. To pass the genetic barrier at aberration frequency 50 percent, there would have to be a comparably enormous random process. This can probably occur only if there are many localities in which colonies of the species are continually becoming extinct to be started anew from single gravid females or at least from a very small number of immigrants (Wright 1940, 1941). As Spurway (1953) has put it "The population size N of Wright should be so small that it may be profitable to think of *some* species originating from a single pair in a new Eden. Being an Adam or Eve gives a monster a chance to hope." She has pointed out that the translocations which distinguish sub-species of the newt, *Triturus cristatus*, in Europe can best be accounted for by supposing that the species reoccupied its range after the Pleistocene by successive crossings of hundreds of mountain passes by single sperm-carrying females, to establish colonies which at first, at least, came wholly from her offspring. The conditions for occasional fixation of a new chromosome pattern would be as great as possible.

INTROGRESSIVE HYBRIDIZATION

The expansion of successful demes into the territories of others, and the consequent formation of new genetic complexes which may tend to displace even the more successful parent deme, is somewhat like the introgressive hybridization of Anderson (1949) except that it is on a finer scale. Anderson stresses the aspect of new variability while I have stressed the selective aspect; but introgressive hybridization can be a significant evolutionary process only if demes that are successful arise from the hybrid swarm. In both interdeme selection and introgressive hybridization, linkage is significant in giving temporary coherence to certain gene complexes (cf. Wright 1931a, p. 146). The principal difference apart from the difference in coarseness is that interdeme selection can operate continuously since differentiation of demes may occur concomitantly with spread of the more successful ones, by means of the joint action of random drift and local mass selection on the variable material arising from the cross-breeding, while introgressive hybridization is presumably a relatively infrequent phenomenon dependent on previous differentiation of species by other processes.

SELECTION AMONG SPECIES AND HIGHER CATEGORIES

Finally, I will touch on the operation of selection in which the objects are at a still higher level than the demes. This is selection among noninterbreeding species and higher categories.

The raw material for such selection is even less random in an absolute sense than in the case of interdeme selection since it consists in the differentiation of these categories that has come about as a result of all of the preceding evolutionary processes. The competing groups may, however, be looked upon as random trials from the standpoint of the course of evolution of life as a whole that comes out of their competition.

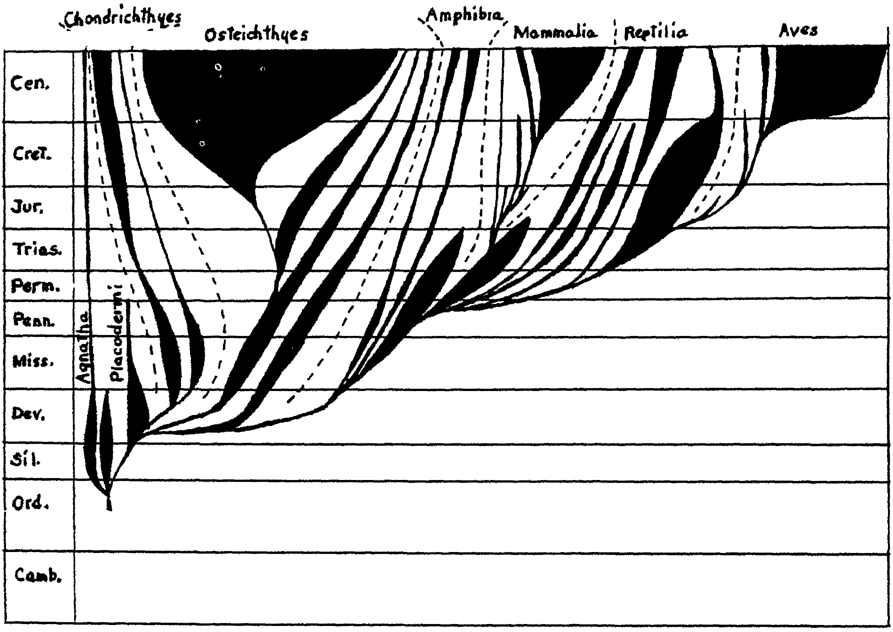


FIGURE 12. The history of vertebrate subclasses illustrating the extent to which the modem families trace to a very small number of forms at the beginning of the Mesozoic.

If there were no selection between such categories, we would expect to find all of those of an early geologic period persisting and all branching to similar extents. Nothing could be farther from the situation that is actually found. (cf. Figure 12 based on Romer 1945). There are some 40,000 species of vertebrates of very diverse types existing now. There was a somewhat comparable diversity and presumably thousands of species at the beginning of the mesozoic. But some 98 percent of the living families may well trace to only about eight of the species of the earlier period (a chondrichthyes ancestor of all sharks and rays, a holostian ancestor of all teleost fishes, a common ancestor of all urodeles and one of all anura, a cotylosaur ancestor (or two) of all turtles, an eosuchian ancestor of all lizards and snakes, a thecodont ancestor of all birds (and perhaps crocodiles), and a therapsid ancestor of all mammals (except perhaps the monotremes).

Probably only about two dozen of the species of vertebrates at the beginning of the mesozoic have left any descendants at all. The rich diversification of the modern vertebrates thus is based on a very limited number of successful types among the many that existed at the earlier period. The course of evolution of vertebrate life and of life in general has been guided throughout by a hierarchy of processes of selection ranging from selection between genes to selection between orders, classes and even phyla.

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THE COMPOSITION OF AN OPEN POLLINATED SEGREGATING COTTON POPULATION

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PURPOSE OF STUDY

The seeds obtained from a field planting of Upland cotton result from a mixture of self and cross fertilization. The relative proportions of self and cross fertilization vary widely in different parts of the American cotton belt; the differences being associated principally with differences in the number and variety of pollen vectors available to effect pollination (Simpson, 1952). Simpson has suggested possibilities of utilizing natural crossing to conserve hybrid vigor by means of new cotton breeding methods and Stephens and Finkner (1953) have pointed out that it may even play a significant though not fully appreciated part in current breeding programs. In the latter paper, methods of estimating the proportion of natural crossing were reviewed and it was suggested that new methods would be necessary to obtain really critical information on the factors involved.

The amount of natural crossing which occurs in a mixture of genotypes under a system of open pollination is dependent not only on the number and activity of pollen vectors but also on the relative proportions in which the genotypes are represented in the common pollen pool. These in turn depend on the relative proportions of genotypes in the initial mixture and on their competitive efficiency. Competitive efficiency may reflect differences between genotypes in respect of numbers of flowers produced, number of pollen grains per flower, and a variety of physiological or mechanical features which might be expected to hinder or favor self-pollination. Very little is known about the relative magnitude of these variables, and in most estimates of natural crossing which have been made their effects have been confounded. It is of some theoretical and practical importance to attempt to devise methods of estimating separately the various factors involved.

The method of measuring natural crossing which has been usually adopted is to plant in isolation a mixture of two stocks, one carrying a seedling marker gene *R*, and the other its recessive allele, *r*. The proportion of *Rr* seedlings in the progenies from open pollinated *rr* plants is considered to be a measure of the amount of natural crossing which has occurred. This assumption should only be valid, of course, if a large excess of *RR* plants is present in the mixed planting, in which case intra-genotypic crossing ($rr \times rr$) can be safely neglected and consequently the *rr* types in the open

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pollinated progeny can be considered to result from self-fertilization. If the proportion of *rr* types in the mixed planting is not negligible, approximate estimates of the amount of natural crossing can be obtained by appropriate weighting of the results obtained. However, the weighting should be based on the relative proportions of *R* and *r* pollen produced in the population (not on the relative proportions of *RR* and *rr* plants) and this can only be estimated very approximately (Finkner, 1954b).

A further disadvantage of the method is the fact that the estimates obtained are only strictly applicable to the stock mixture employed in the mixed planting. Differences between stocks in respect of time of anthesis, relative pollen abundance, relative pollen establishment etc., may bias the results obtained and make their general application of doubtful validity. By employing mixed planting of three stocks, differentiated by three contrasting seedling marker genes, Finkner (loc. cit.) showed clearly that stock differences of this type cannot be safely ignored. The estimates he obtained from the same mixed planting varied from 40% to 70% natural crossing depending on the stock combinations tested. He also obtained evidence of interaction between stock combination and locality in replicated experiments.

From the genetic point of view the apparent proportion of natural crossing in a mechanical mixture of stocks is dependent on the total genotypic differences between the stocks concerned insofar as these affect "crossability," and more specifically on pleiotropic effects of the seedling marker genes used to furnish the estimates on which it is based. In a mechanical mixture the "general" and "specific" effects are confounded and their relative importances cannot be evaluated. For this reason Stephens and Finkner (1953) suggested that estimates of natural crossing might be based more profitably on segregating populations, particularly in populations segregating concurrently for several seedling marker genes. In such material "general" genotypic differences should be segregating at random and hence the resulting differences should be neutralized. "Specific" differences associated with the seedling markers themselves could therefore be compared, pleiotropic effects analyzed, and estimates of the precision of the method obtained. An experiment of this type will be reported in this paper.

MATERIALS

The two parent stocks which provided material for analysis were contrasted in respect of four seedling marker genes as follows:

Parent A	*Parent B*
Red stem -*R*	Green stem -*r*
Non-yellow leaf -*V*	Yellow leaf -*v*
Normal leaf -*Cr*	Crinkle leaf -*cr*
Narrow leaf -*L*	Broad leaf -*l*

All Upland stocks which are grown commercially have the genotype, *rr, VV, CrCr, ll*. The parents listed above, therefore, include two dominant and two recessive mutants as compared with the "normal" type.

F_2 seeds obtained from a cross between the parent types were planted in the greenhouse in small paper cups. Over 95% germination was obtained. When the seedlings were well established they were transplanted to an isolation plot in the field. This was situated in the middle of a corn field surrounded by woodland on three sides and a tobacco field on the fourth side. It was therefore well isolated from extraneous sources of cotton pollen. The seedlings were planted in three rows, each row constituting a replicate in the subsequent analysis. A total of 663 seedlings was established, of which only two died early or failed to produce flowers.

All surviving plants were scored completely for the four seedling markers concerned. The proportions obtained were consistent with normal mendelian expectation except in the case of the *Cr* locus, where there was a significant deficiency in the recessive class (Table 1). When the experiment was begun it was thought that all the loci were independent but later it was discovered that *L* and *Cr* were loosely linked (circa 42% crossing over) and this fact has to be borne in mind in interpreting the results.

TABLE 1

SINGLE GENE RATIOS OBTAINED IN A F_2 POPULATION SEGREGATING FOR FOUR SEEDLING MARKERS

	Cr -	*crcr*	*R -*	*r r*	*L -*	*l l*	*V -*	*v v*
Freq.	531	130	515	146	480	181	506	155
χ^2	10.026		2.990		2.002		0.848	
P	.01-.001		.10-.05		.20-.10		.50-.30	

When the scoring was completed, plants having the following genotypes were selected as female parents for progeny analysis:

R -, L -, cr cr, v v
r r, l l, Cr -, V -
R -, l l, Cr -, v v
r r, L -, cr cr, V -
R -, l l, cr cr, V -
r r, L -, Cr -, v v

It will be noted that these six types represent every possible combination of doubly recessive genotypes. In order to obtain adequate sized progenies for testing it was necessary to pool the open pollinated seeds from plants with the same genotype. In each of the three rows the seeds from similar genotypes were pooled separately. In each case the pooled progeny was obtained from not less than three nor more than five plants. Progeny analysis was therefore based on a total of eighteen pooled progenies (3 rows with 6 genotypes per row). It was desirable to have some check on the accuracy of sampling, so triplicate samples of seeds from each pooled progeny were drawn and the seedling characters were scored separately for each sample. All seeds were acid-delinted and planted in small greenhouse beds.

METHODS OF ANALYSIS

Because the term "natural crossing" tends to be used rather loosely it will be convenient to introduce a more precise definition for use in these studies. Consider the six doubly recessive genotypes selected as female parents and listed in the previous section. These have the general genotypic formula, *aabb*. Their open-pollinated progeny will consist of four genotypes, *AaBb, Aabb, aaBb,* and *aabb*. The sum of the first three classes (excluding *aabb*) as a proportion of the total progeny represents the proportion of apparent out-pollination (AOP). The fourth class, *aabb*, results from a mixture of self and intra-genotypic pollination. The proportion of apparent out-pollination plus the proportion of intra-genotypic pollination represents the total proportion of out-pollination (TOP) which provides a measure of the total activity of the pollen vector and its contribution toward the setting of the crop. Providing the genetic markers employed were selectively neutral and that their frequencies in the F_2 population were strictly in accordance with mendelian expectation, one would expect that there would be no significant differences between the AOP estimates obtained from the six different genotypes used as female parents. Furthermore, all the classes included in the AOP estimates (*AaBb, Aabb, aaBb*) should be equal in size. Under these conditions an estimate of TOP could be obtained directly from AOP by simple weighting (TOP = 4/3 × AOP). However, as shown in table 2, the proportions of the out-pollinated classes deviated from mendelian expectation, so that the estimation of TOP had to be calculated from the actual gene frequencies as will be shown later.

From these considerations it should be clear that any significant differences between the TOP estimates obtained from the six different genotypes used as female parents should be attributable to pleiotropic effects of the marker genes concerned on female crossability. Also any significant departures from expectation, based on gene frequencies, in the classes included in the AOP estimates should be due to pleiotropic effects of the markers concerned on pollen production and/or pollen efficiency.

In the experiments to be described it is assumed that the pollen vectors (chiefly bumble bees) work at random. Previous work at this station (Stephens and Finkner, 1953, Finkner, 1954a) is in accordance with this assumption. At the same time it is realized that a "normal" system of pollination would not necessarily hold for areas where the proportion of natural crossing is low or where different species of vector are active. It is possible, for instance, that where natural crossing is low, flower visitation by the vector might be more in accordance with a Poisson than a normal distribution. It is also possible that honey bees, which are important in some areas, might discriminate between different genotypes. A little consideration shows, however, that the latter situation would not be expected to introduce a bias—it would merely reduce the proportion of apparent out-pollination in *all* genotypes. In Raleigh, natural crossing is obviously extremely high, field observation shows that all flowers are repeatedly visited during the morning hours; and experimental data are available which suggests that

TABLE 2

SEGREGATIONS OBTAINED IN THE OPEN POLLINATED PROGENIES OF SIX DOUBLY RECESSIVE GENOTYPES SELECTED FROM AN ISOLATED F_2 POPULATION. EACH GENOTYPE IS REPLICATED IN THREE ROWS (SEE TEXT). THE FINAL COLUMN, AOP, IS THE APPARENT PROPORTION OF OUT-POLLINATION EXPRESSED IN PERCENT.

(A) Parent genotype: R-L- cr cr vv

Row	*Cr V*	*Cr v*	*cr V*	*cr v*	*Total*	*AOP*
1	27	57	17	33	134	75.37
2	33	35	15	21	104	79.81
3	19	37	17	57	130	56.15
	79	129	49	111	368	....

(B) Parent genotype: rr ll Cr-V-

Row	*R L*	*R l*	*r L*	*r l*	*Total*	*AOP*
1	21	24	51	130	226	42.48
2	17	35	38	148	238	37.82
3	20	52	23	175	270	35.19
	58	111	112	453	734	....

(C) Parent genotype: R-ll cr cr V-

Row	*L Cr*	*L cr*	*l Cr*	*l cr*	*Total*	*AOP*
1	41	31	57	78	207	62.32
2	39	24	40	25	128	80.47
3	12	11	13	14	50	72.00
	92	66	110	117	385	....

(D) Parent genotype: rr L-Cr-vv

Row	*R V*	*R v*	*r V*	*r v*	*Total*	*AOP*
1	9	7	8	37	61	39.34
2	32	25	25	118	200	41.00
3	13	15	25	97	150	35.33
	54	47	58	252	411	....

(E) Parent genotype: R-ll Cr- vv

Row	*L V*	*L v*	*l V*	*l v*	*Total*	*AOP*
1	34	34	29	97	194	50.00
2	25	23	36	79	163	51.53
3	24	22	43	113	202	44.06
	83	79	108	289	559	....

(F) Parent genotype: rr L- cr cr V-

Row	*R Cr*	*R cr*	*r Cr*	*r cr*	*Total*	*AOP*
1	15	12	30	28	85	67.06
2	40	21	65	67	193	65.28
3	28	17	100	101	246	58.94
	83	50	195	196	524	....

pollen applied to a cotton stigma two hours later than a prior excess pollination contributes to fertilization (Finkner, 1954c). This combination of circumstances might be expected to result in an effectively random system of pollination.

EXPERIMENTAL RESULTS

As explained earlier in this paper, the eighteen pooled progenies available for analysis were each divided into triplicate samples which were planted out in greenhouse beds in October, December and March successively. Contingency tests showed no significant heterogeneity between samples from the same pooled progeny except in the case of those progenies which were segregating for the Crinkle gene (*cr*). Further analysis showed the departure from homogeneity within these progenies was entirely attributable to a deficiency of Crinkle plants in the December planting, the segregation of other genes in the same progenies being homogeneous between samples. Previous experience with greenhouse plantings in the winter months has made it evident that under such conditions the penetrance of the Crinkle gene is much reduced, but it had not been anticipated that the reduction would be of such magnitude as to vitiate accurate scoring. However, since the heterogeneity between samples was confined to the December planting of those progenies which were segregating for Crinkle, it seemed permissable to exclude them from the experimental analysis and to pool all samples from the remaining progenies. Consequently, the segregations summarized in Table 2 represent the pooled data from triplicate samples in all progenies not segregating for Crinkle, (genotypes B, D and E) and the pooled data from *duplicate* samples in those progenies which have Crinkle segregates (genotypes A, C and F).

(1) *Apparent out-pollination* (AOP)

The first point of interest to be considered in table 2 is the apparent proportion of out-pollination (AOP) which is shown in the final column in each segregation. It is evident that the AOP estimate varies considerably according to the genotypic segregation on which it is based. In general the AOP estimate is high when determined from the open pollinated progenies of plants homozygous for Crinkle (genotypes A, C and F). Since the AOP

TABLE 3.

ANALYSIS OF VARIANCE OF THE AOP ESTIMATES GIVEN IN TABLE 2.

	D.F.	Sum. sqq.	Variance	"F"	
Rows	2	251.8902	125.9451	3.69	insig.
Genotypes	5	3524.9060	704.9812	20.66	<.001
Cr v cr 1		3217.3568	3217.3568	94.30	v. low
Residual 4		307.5492	76.8898	2.25	insig.
Error	10	341.1993	34.1199	....	
Total	17	4117.9955	....	....	

Mean apparent out-pollination

Genotypes:	*l cr*	*cr v*	*r cr*	*l v*	*r v*	*r l*	S.E.
	71.60	70.44	63.76	48.53	38.56	38.50	±3.37
		68.60			41.86		

estimates all lie within the range of 35 per cent to 80 per cent it is permissable to apply an analysis of variance to the percentage estimates without transforming the data. The results of this analysis are presented in table 3. The analysis shows that the AOP estimates determined from different rows in the experimental plot do not differ significantly, which may be taken to lend some support to Finkner's (1954a) finding that natural crossing under Raleigh conditions is effectively random. On the other hand the AOP estimates obtained from different genotypes show very large and highly significant differences. The inter-genotypic variance is further analysed into two parts (1) attributable to differences between *Cr*- and *cr*-containing genotypes and (2) a residual component which is a composite of all other genotypic differences under test. It is clear that the variation in AOP estimates is almost entirely due to the high proportion of apparent out-pollination obtained from Crinkle plants as compared with other genotypes. Crinkle genotypes on the average show an apparent out-pollination of 68.60 per cent as compared with 41.86 per cent for Non-Crinkle genotypes.

(2) *Composition of the pollen "pool"*

The second point of interest in table 2 is the marked deviation from equality which occurs in most of the out-pollinated classes (i.e. the classes listed in the first three columns in each segregation). These classes should occur in a 1:1:1 ratio if the gene frequencies in the original population were in accordance with mendelian expectation and if all genotypic combinations in the male gametes had equal viabilities. It will be noted that there seems to be a general tendency for those classes which contain double mutant combinations (*R L* in B, *L cr* in C, *R v* in D, *L V* in E and *R cr* in F) to be small. The double mutant class in A is the double recessive class, *cr v*, which presumably includes selfed and out-pollinated progeny, and hence cannot be compared with the other double mutant classes.

The out-pollinated classes in each row provide two estimates of the relative frequency of a normal gene and its mutant allele. Thus in table 2, Genotype A, Row 1, a comparison of the classes *Cr V* and *Cr v* gives a measure of the relative contribution of *V* and *v* pollen and, similarly, a comparison of classes *Cr V* and *cr V* measures the relative contribution of *Cr* and *cr* pollen. Altogether the data in table 2 provide nine estimates of the relative contributions of each allelic pair.

Contingency tests of the estimates so obtained were made to determine (a) if the four allelic pairs - *Cr*:*cr*, *R*:*r*, *L*:*l* and *V*:*v* occurred in different ratios (b) if ratios obtained for each allelic pair were influenced by the family or row from which they were obtained. The results of these tests are summarized in table 4. In this table the total heterogeneity is first partitioned into a portion due to differences between allelic pairs with 3 degrees of freedom and a portion within allelic pairs with $4 \times 8 = 32$ degrees of freedom. Both items show considerable heterogeneity, but as shown in the table, if the mean squares obtained from the χ^2 values are compared in an "F" test, the "between pair" effects are much greater than the "within pair" effects. It may be concluded that most of the variation is due to the fact that the four allelic pairs occur in different ratios. The pooled data for each of the four ratios is shown at the foot of table 4.

The heterogeneity "within pairs" can then be partitioned for each allelic pair separately into 3 components ascribable to differences between (a) families (b) rows and (c) family × row interactions. This partitioning shows that the ratios of two of the allelic pairs, *Cr*:*cr* and *L*:*l*, are homogeneous. *R*:*r* ratios are heterogeneous; apparently showing significant differences

TABLE 4.

CONTINGENCY TESTS OF RATIOS OBTAINED IN THE OUTPOLLINATED CLASSES SHOWN IN TABLE 2.

Source of heterogeneity	D.F.	χ^2	Mean Square	F
Between allelic pairs	3	67.73***	22.8	11.64***
Within allelic pairs				
(a) *Cr*: *cr*				
Families	2	0.64	0.32	1.07
Rows	2	2.52	1.26	4.20
Fams × rows	4	1.21	0.30	....
(b) *R* : *r*				
Families	2	11.90**	5.95	2.56
Rows	2	6.69*	3.35	1.44
Fams × rows	4	9.26	2.32	....
(c) L : l				
Families	2	5.25	2.63	2.63
Rows	2	5.89	2.95	2.95
Fams × rows	4	3.99	1.00	....
(d) *V*: *v*				
Families	2	9.44**	4.72	15.73*
Rows	2	4.23	2.12	7.06*
Fams × rows	4	1.21	0.30	....
Total	32	62.23***	1.94	
Grand total	35	129.96***		

*significant at 5% level; ** at 1% level; *** at 0.1% level.

	Cr	*cr*	*R*	*r*	*L*	*l*	*V*	*v*
Pooled data for each allelic pair	254	165	195	365	233	329	216	255
Mutant class as percent of total	39.38		34.82		41.46		54.14	

between families and between rows. However, when these components are compared with the family × row interaction component in an "F" test, the differences are not significant. This means that although the *R*:*r* ratios show considerable variation from sample to sample this variation cannot be ascribed with confidence to differences between families nor differences between rows *per se*. In the case of the *V*:*v* ratios, the appropriate χ^2 and "F" tests show that there are significant differences between families and that both family and row effects are significantly greater than effects due to family × row interaction.

Considering the data as a whole it may be concluded that the alleles in each pair occur with very different relative frequencies, and that although the ratio of a gene to its mutant allele is more variable than can be ascribed to chance sampling error, such variation is relatively low in magnitude and not consistently associated with family or row differences.

The combined data from each inter-allelic comparison show that there are large deviations from equality except in the case of the *V*:*v* comparison. in the other three comparisons the mutant classes *cr*, *R* and *L* are markedly smaller than the corresponding normal classes. Three factors might contribute to these deviations, (1) unequal frequencies in the initial F_2 population (2) unequal production of pollen by different genotypes (3) selective advantages of normal over mutant pollen. The contribution of the first factor can be estimated by comparing the expected gene frequencies in the F_2 population with their observed frequencies in the out-pollinated classes in table 4. The expected frequencies can be estimated from the F_2 segregations shown in table 1 where it can be seen that one of the segregations (*Cr*:*cr*) differs markedly from mendelian expectation. If all the genotypes in the initial F_2 population contributed equally to the common pool of pollen then the expected gene frequencies could be calculated in the following manner: From the data given in table 1 the proportion of *rr* genotypes is 146/661 or 0.2208775. The frequency of *r* pollen in the population is thus expected to be the square root of this value or 0.470 and the frequency of *R* will be 1 – 0.470 or 0.530. The expected frequencies of the other three allelic pairs can be calculated similarly. The actual gene frequencies in table 4 can then be fitted to the calculated frequencies as shown in table 5. Agreement between the two sets of data is very poor. Three of the four mutant genes, *cr*, *L* and *R*, appear less frequently in the pollen pool than would have been expected from the composition of the F_2 population. On the other hand, the mutant gene, *v*, appears more frequently than expected. Clearly the composition of the initial population of plants gives little indication of the relative composition of the pollen which it produces.

TABLE 5.

COMPARISON OF OBSERVED GENE FREQUENCIES GIVEN IN TABLE 4 WITH THOSE EXPECTED FROM THE COMPOSITION OF THE F^2 POPULATION (CALCULATED FROM DATA IN TABLE 1).

	Cr	*cr*	*R*	*r*	*L*	*l*	*V*	*v*
Obs.	254	165	195	365	233	329	216	255
Exp.	233	186	297	263	268	294	244	227
χ^2	4.264		74.589		8.738		6.667	
P	.05-.02		<.001		.01-.001		.01-.001	

The second factor which might be important in determining the composition of the pollen pool is the relative production of pollen by different genotypes.

If mutant types produced less flowers or less pollen per flower than their normal sibs, one would expect a deficiency of mutant genes in the pollen pool. Some independent evidence obtained by Mr. J. C. Williams at this station (data unpublished) is of interest in this connection. He finds that the open pollinated progeny from a population segregating for the same *L* and *R* mutants shows a marked shift in frequency in favor of their normal alleles. Again, unpublished data of Mr. S. Cain at College Station, Texas, showed that the mutant segregates, *LL* and *RR* from the F_2 population yielded fewer bolls (and hence fewer flowers) per plant than their normal sibs. In a competitive system, therefore, shifts in favor of the normal genotype are to be expected and it is surprising that in the present experiment the frequency of the *v* mutant is shifted in the opposite direction.

The third factor which requires consideration is pollen competition. If this were an important factor selective differentials would be established between pollens of different genotypes, and the gene frequencies would vary accordingly. The four mutants used in this experiment have all been studied repeatedly in test crosses by several workers and no consistent deviations from expected mendelian ratios have been reported. If pollen competition were important one might expect that the segregations obtained would follow no consistent pattern—in fact the establishment of a mendelian ratio is good presumptive evidence that pollen competition is an unimportant factor in so far as the genes tested are concerned. For this reason it is not believed that the heterogeneity "within allelic pairs" in table 4 can be attributed to pollen competition. A more likely interpretation is that the quantity of pollen produced depends on the genotypic composition of the plant on which it is borne. Thus although pollen vectors might work at random from row to row through the experimental plot, and as a consequence the AOP estimates would not show significant row differences (table 3), yet the precise composition of the outpollinated classes might reflect local variations in the genotypic compositions of plants in the neighborhood of those sampled. Such variations however are not consistent, nor do they play a major role in determining the average composition of the open-pollinated population.

(3) *Total out-pollination (TOP)*

The genetic composition of the common pollen pool will naturally affect the AOP estimates obtained. A genotype which contributes relatively less to the pool should have less opportunity for intra-genotypic crossing and consequently more of the out-pollination it receives will be from "foreign" genotypes. In order to arrive at a useful estimate of total out-pollination it is necessary to eliminate the effects of different gene frequencies. In a random system a change in gene frequency leading to an increased amount of foreign pollination (AOP) should be exactly balanced by a decreased amount of intra-genotypic pollination, so that the total out-pollination which includes both, should remain constant.

The procedure used to estimate total out-pollination (TOP) is to calculate from the actual gene frequencies the expected amount of intra-genotypic pollination and to adjust the AOP estimates accordingly. Consider the out-pollination of Genotype A in table 2. The single gene ratios are *Cr*: *cr* and *V*: *v*. The best estimates of the relative proportions of these alleles in the population are the mean percentages given in table 4. These are 39.38 per cent for *cr* and 54.14 per cent for *v*. Gametes carrying the combination *cr v* are therefore expected to occur with a frequency equal to the product of the individual gene frequencies (i.e. 39.38% × 54.14%, or 21.32%). The out-pollinated classes, *Cr V*, *Cr v* and *cr V* should therefore account for (100 − 21.32%) of the total out-pollination or 78.68 per cent. It follows that the AOP estimate for Genotype *cr v* of 70.44% which is given in table 3 only measures 78.68 per cent of the total out-pollination. Total out-pollination (TOP) should therefore amount to $\frac{100 \times 70.44}{78.68}$ or 89.53 per cent. TOP estimates for all six genotypes can be calculated in similar fashion except that the estimate for *l cr* has to be weighted slightly to allow for the coupling linkage between *l* and *cr*. The TOP estimates so obtained are given in table 6. Their values indicate that most of the seeds set on genotypes homozygous for Crinkle can be attributed to out-pollination, the average estimate for the three Crinkle genotypes being over 90 per cent. On the contrary the Non-Crinkle genotypes give an average estimate of about 64 per cent.

TABLE 6.

ESTIMATED TOTAL PROPORTION OF OUT-POLLINATION (TOP) IN PERCENT FOR THE SIX GENOTYPES LISTED IN TABLE 2.

Genotypes:	*l cr*	*cr v*	*r cr*	*l v*	*r v*	*r l*
TOP %	97.12	89.53	85.78	71.04	59.59	62.26
		90.81			64.30	

(4) *The relation between foreign pollination (AOP), intra-genotypic pollination and selfing.*

The compositions of the open pollinated progenies of the six genotypes under test are summarized in table 7. In this table the frequencies of the same genotypes in the common pollen pool (calculated from the data in table 4) are presented for comparison.

The data show, as expected, that there is a strong negative correlation between the AOP and selfed fractions associated with the different genotypes. A more surprising feature is that the intra-genotypic fraction tends to remain constant over all genotypes. The reason for this situation can be best appreciated from a general theoretical consideration. For any given genotype, let s be the proportion of self-pollination it receives, and let f be its relative pollen output (i.e. the proportion by which it is represented in the common pollen pool). The expected composition of its open pollinated progeny will then be as shown in the rightmost column of table 7. The

TABLE 7.

SUMMARY OF PERCENTAGE COMPOSITION OF THE PROGENIES OBTAINED BY OPEN POLLINATION OF THE SIX GENOTYPES. FREQUENCIES OF THE SAME GENOTYPES IN THE POLLEN POOL ARE INCLUDED FOR COMPARISON.

Genotypes	*l cr*	*cr v*	*r cr*	*l v*	*r v*	*r l*	General
AOP	71.60	70.44	63.76	48.53	38.56	38.50	(l-s) (l-f)
Intra-genotypic	25.52	19.09	22.02	22.51	21.03	23.76	(l-s)f
Selfed	2.88	10.47	14.22	28.96	40.41	37.74	s
Total	100.00	100.00	100.00	100.00	100.00	100.00	1
Frequency in pollen pool	26.27*	21.32	25.67	31.69	35.29	38.16	f

*Adjusted for coupling between *L* and *Cr*.

expected proportion resulting from intra-genotypic pollination is $(1 - s)\,f$: a value which would obviously tend to remain constant if s and f were positively correlated. There is in fact a significant correlation between the actual s and f values in table 7 ($r = +0.8966$; $P = .02$-$.01$).

A rather simple explanation can be offered for this correlation. The relative pollen output (f) of a genotype should depend primarily on the number of flowers produced and the number of pollen grains per flower. The relative amount of selfing (s) should be unaffected by the number of flowers produced, but one would expect that a flower which produced abundant pollen would be self-pollinated to a greater degree than a flower which produced relatively little pollen. Both s and f therefore should be dependent on a common variable—the amount of pollen produced per flower. The genotypes which produce the largest amount of pollen per flower should self most, contribute most to the pollen pool and receive the least amount of foreign pollen. Intra-genotypic pollination should remain relatively unaffected. Reference to table 7 shows that most of the differences between genotypes can be interpreted satisfactorily on this basis. On the other hand, if variation in number of flowers produced were the more important factor in determining the compositions of the progenies from the different genotypes, the correlation between s and f should be obscured, and the intra-genotypic fraction instead of remaining constant should vary inversely with the AOP fraction. The data in table 7 lend no support to this alternative interpretation.

DISCUSSION

The analysis of natural crossing in a segregating population makes possible the separate estimation of some of its components.

(1) *Vector activity*

The estimates of total out-pollination (TOP) given in table 6 are independent of relative gene frequencies in the population and thus they provide an unbiased estimate of the total vector activity under the conditions of the experiment. Over half of the seeds set in all four genotypes tested resulted from out-pollination, the actual estimates ranging from 59.59% to 97.12%. In general, genotypes homozygous for Crinkle showed the highest percentage of out-pollination.

Estimates of total out-pollination in Non-Crinkle types average 64.30 per cent, a figure which agrees reasonably well with those obtained earlier by Finkner (1954b). He used mechanical mixtures of stocks in a randomized system, but attempted to adjust his estimates in accordance with the expected composition of the pollen population. His estimates (in Raleigh) for out-pollination in his Virescent Yellow (*v*) stock were of the order of 67% which may be compared with 71.04% (*l v*) and 59.59% (*r v*) in table 6. Estimates for his Green Leaf stock averaged 45%, which may be compared with 62.26% for *r l* in table 6. The discrepancy here can most likely be attributed to the fact that the design of Finkner's experiment only allowed him to estimate out-crossing of his Green Leaf stock with *R* pollen. The relatively low production of *R* pollen as shown in table 4 would be expected to give an underestimate in his experiment.

On the whole these data suggest that as much as sixty per cent of the seed set by a cotton population in the Raleigh area may result from out-pollination by the local vectors (chiefly bumble bees). If this situation is typical for commercial plantings of cotton in this area one would suspect that the activity of the vectors may play an important part in the determination of yield. It has been commonly observed that the yield of cotton from self-pollinated bolls is less than from open-pollinated bolls. The difference has usually been attributed to the deleterious effect of bagging or other mechanical devices used in selfing. The question may perhaps be re-opened as to whether the potential yield of a cotton plant is practically attainable in the absence of a thriving population of pollen vectors. Some data obtained by Loden and co-workers (1950) for another purpose are of interest in this connection. They found that pre-pollination of unreceptive stigmas, followed by a second application of pollen at the normal time, gave an increase in seed-set of about 30% as compared with their controls. Three controls were used—"normally" selfed, hand-crossed, and open-pollinated flowers—and no significant differences between them were found. These experiments were carried out in Texas where natural crossing is very low. An obvious corollary of their results is the conclusion that if the pre-pollination technique was capable of increasing seed-set by this order of magnitude, then none of the control methods could have approached achieving the full seed setting capacity of their plants. It would seem that selfing, crossing and exposure to a low population of vectors might be equally inefficient in producing a full seed-set, as compared with the accomplishments of an active and adequately sized population of bumble bees.

(2) *The effective gene frequencies in the pollen pool*

The amount of hybridization which occurs in an open pollinated cotton population is dependent not only on the vector activity but also on the effective gene frequencies in the pollen pool. The best estimates of effective gene frequencies are the mean gene frequencies in the out-pollinated classes which are shown in table 4.

The effective gene frequencies are in turn dependent on four possible factors:

(a) The relative proportions of genotypes in the initial plant population.
(b) The relative number of flowers produced by each genotype.
(c) The relative number of pollen grains per flower.
(d) The competitive efficiency of pollens with different genetic constitutions.

The evidence which has been presented shows that the factors of chief importance are (a) and (c) above. The effective gene frequencies in the pollen pool are not directly proportional to the relative composition of the initial plant population, because different genotypes contribute unequally to the common pollen pool. The substitution of a single normal gene by its mutant allele is sufficient to alter the pollen output considerably. Three of the mutants tested, *cr, R* and *L,* reduced pollen output: the fourth, *v,* increased it. In general one would expect mutant "markers" to depress yield. An important component of yield is the number of flowers produced, and one might expect that reduced pollen output in mutants would be determined primarily by this factor. However the composition of the progenies resulting from open pollination in this experiment (table 7) suggests that the amount of pollen produced per flower may be more important than the number of flowers produced per genotype. Differences between genotypes in respect of the amount of pollen produced per flower would account for the positive correlation between relative pollen output and degree of selfing, the constancy of the proportion of intra-genotypic pollination, and the negative correlation between the proportion of foreign pollination (AOP) and selfing.

There was no indication that the genetic constitution of the pollen affects its competitive efficiency. Previous work has indicated that the markers used segregated in normal mendelian ratios, and in the present experiment their relative frequencies in the open pollinated progenies were not consistently affected by the genetic composition of the families in which they were recovered (table 4).

The general picture which emerges from these studies is that of a randomly operating population of vectors whose effects on the genetic composition of the plant population in which they work are largely determined by the mechanical interaction of three factors, (1) the number of vectors (2) the relative proportions of genotypes (3) the number of pollen grains produced per flower. While it is certain that this picture is oversimplified and subject to modification by other variables (e.g. variations in numbers of flowers per genotype, in time of flowering and possible in flower structure it is probably representative of the actual situation in a segregating population, where many of the minor factors would be distributed at random.

SUMMARY

(1) By analysing the open pollinated progeny from a segregating population of cotton whose initial composition in respect of four marker genes is known,

it has been possible to estimate separately the relative frequency of three types of pollination:

(a) The proportion of apparent out-pollination (AOP), i.e. "foreign" pollination.

(b) The proportion of intra-genotypic pollination, i.e. involving different flowers of the same genotype.

(c) The proportion of self-pollination (i.e. within the same flower). The sum of (a) and (b) is the total out-pollination (TOP). It is independent of the gene frequencies in the population and therefore provides unbiased estimates of the total vector activity.

(2) The amount of hybridization which occurs depends not only on the number and activity of the vectors, but also on the effective gene frequency in the population. The latter is shown to depend primarily on (a) the relative frequencies of genotypes in the initial population (b) the relative amount of pollen produced *per flower.*

(3) The amount of pollen per flower apparently is of major importance not only in determining the amount of selfing but also the amount of pollen contributed to the pollen pool, because these two estimates, as determined from the data, are highly correlated. As a result of this (positive) correlation it is shown that the proportion of intra-genotypic pollination remains relatively constant for all genotypes.

(4) Under the conditions of the experiment vector activity was extraordinarily high, resulting in total out-pollination estimates of 60 to 90 per cent, depending on the genotype involved. It is suggested that vector activity of this magnitude may be an important though overlooked factor in determining the yield of the crop.

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CROSSING OVER AND INVERSIONS IN COADAPTED SYSTEMS*

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Wallace (1953) has recently proposed that combinations of certain chromosomal inversions will be rare in natural populations of *Drosophila pseudoobscura*; namely, combinations of simple inversions between which crossing over could occur. Such combinations of gene arrangements, taken in groups of three, have been termed *triads* by Wallace. Crossing over between these inversions is suggested as a means whereby the integrity of favorable or so-called coadapted gene combinations would be lost. For example, in populations of *D. pseudoobscura* the inversions known as Arrowhead (AR) and Pikes Peak (PP) would form a triad in combination with the Standard (ST) gene arrangement.

Since both AR and PP are simple inversions with respect to ST it is assumed that sufficient crossing over would occur between each of them and ST so as to permit the transfer of genes between all three chromosome types. Thus, Wallace states that double crossovers between ST and PP would prevent the maintenance of coadapted systems. On the other hand a combination of ST, AR, and CH (Chiricahua) is not an unexpected one in populations of this species since CH is a complex, overlapping inversion with respect to both ST and AR. Figure 1 represents the gene arrangements of AR, CH, and PP with respect to ST.

Wallace's hypothesis is based upon the fact, discovered in *D. melanogaster* by Sturtevant (1919), that crossing over is largely suppressed in inversion heterozygotes. Dobzhansky and Epling (1948), studying the same phenomenon in *D. pseudoobscura*, observed that crossing over was more highly suppressed among inversion heterozygotes involving complex, overlapping gene arrangements such as CH in combination with ST, than in the case of the simple AR inversion when in combination with ST. Thus, they concluded that inversion is a means whereby combinations of genes imparting a favorable adaptive character to their carriers can be retained without loss through recombination due to crossing over. This conclusion and the triad hypothesis of Wallace assumes logically that the complex inversion heterozygotes, because of conditions of their pairing, will show a suppression of crossing over greater than that exhibited by the simple inversions. The triad hypothesis depends to a large extent upon the assumption that a direct relationship exists between the cytological configuration of pairing in the salivary gland chromosomes of inversion heterozygotes and the genetic consequences of crossing over. The recent observations

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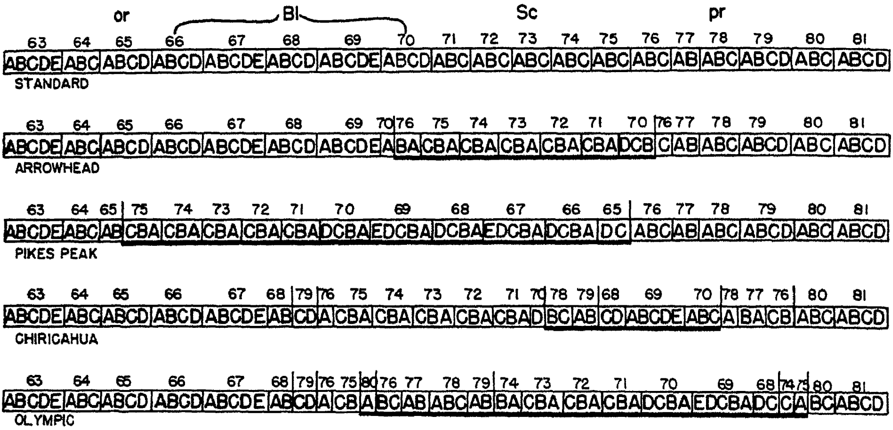

FIGURE 1. The Standard, Arrowhead, Pikes Peak, Chiricahua, and Olympic third chromosome gene arrangements in *Drosophila pseudoobscura*. (from Dobzhansky and Epling, 1944). The positions of the mutant loci *or*, *Bl*, *Sc*, and *pr* as determined by Tan (1937).)

of Dobzhansky (1952) and Epling (unpublished) that the frequency of the PP gene arrangement has increased significantly in certain populations of *D. pseudoobscura* are of interest in this regard. The populations studied by Dobzhansky and Epling are characterized by a relatively high frequency of both the ST and AR gene arrangements. The observation of increasing PP frequency is an unexpected one in terms of the hypothesis proposed by Wallace. In addition a new simple inversion has been found in San Jacinto populations of *D. pseudoobscura* (Epling, unpublished) which forms a triad with ST and AR. In view of these observations, it is therefore highly desirable to obtain genetic tests of the triad hypothesis. Accordingly, this paper presents data derived from the genetic analysis of crossing over in a variety of *D. pseudoobscura* third chromosome inversions as they relate to Wallace's triad hypothesis.

MATERIALS AND METHODS

Only the Standard (ST) third chromosome gene arrangement bears the markers suitable for crossover analyses; these are orange eye (*or*), Blade wings (*Bl*), Scute bristles (*Sc*), and purple eye (*pr*). The location of these markers relative to different inversions is given in figure 1. Crossing over has been studied between the marked ST chromosome and the wild type third chromosome gene arrangements known as AR, CH, OL (Olympic), PP, and VF (Vandeventer Flat). These inversions, with the exception of VF, are described in Dobzhansky and Epling (1944). VF is the arrangement recently discovered by Epling; it will be described shortly by him. It is to be noted that AR, PP, and VF are simple inversions with respect to ST while CH and OL are complex, overlapping inversions.

The experimental procedures followed in this study are identical with previously described analyses of crossing over in *D. pseudoobscura* as carried out in this laboratory (Levine and Levine 1954, 1955).

TABLE 1

CROSSING OVER BETWEEN THE *or Bl Sc pr* (ST) CHROMOSOME AND VARIOUS WILD TYPE THIRD CHROMOSOMES

		ST	*AR*	*PP*	*VF*	*CH*	*OL*
0	+	997	2136	2877	1082	2576	682
	or Bl Sc pr	622	1870	3227	1404	1774	848
1	*or*	117	54	0	224	22	2
	Bl Sc pr	118	70	0	204	18	6
2	*or Bl*	338	20	0	273	0	0
	Sc pr	254	16	0	237	3	0
3	*or Bl Sc*	444	0	0	32	0	0
	pr	362	10	0	27	2	0
1-2	*Bl*	6	0	0	4	0	0
	or Sc pr	5	1	0	1	0	0
1-3	*Bl Sc*	69	0	1	2	0	0
	or pr	42	0	0	0	0	0
2-3	*Sc*	88	0	0	2	0	0
	or Bl pr	51	2	0	0	0	1
1-2-3	*or Sc*	0	0	0	0	0	0
	Bl pr	0	0	0	0	0	0
Total		3513	4170	6105	3492	4395	1539

TABLE 2

PER CENT CROSSING OVER IN THE *or Bl Sc pr* REGION OF THE THIRD CHROMOSOME

	ST	AR	PP	VF	CH	OL	TL*
or-Bl	10.16	2.99	0.016	12.46	0.91	0.52	0.2
Bl-Sc	21.12	0.93	0	14.81	0.06	0.06	0
Sc-pr	30.06	0.23	0.016	1.80	0.04	0.06	0

*Data of Dobzhansky and Epling (1948).

RESULTS

The data are summarized in tables 1 and 2. It must be pointed out that there is significant variation in crossing over between different strains of ST and AR. The nature of this variation is discussed elsewhere (Levine and Levine 1955). From tables 1 and 2 it will be seen that crossing over in the complex, overlapping inversions (CH, OL, and TL) is highly suppressed while the simple inversions (AR, PP, and VF) have various effects on crossing over. It is seen that, compared with ST, VF has suppressed crossing over in the *Sc-pr* region only. The AR inversion shows suppression over the three regions while only one crossover out of 6105 flies was observed with the PP chromosome.

DISCUSSION

The results of the experiment reported here as regards the PP gene arrangement are of particular interest. They reveal, by virtue of almost complete suppression of crossing over in the ST/PP heterozygote, a basis for the possible maintenance of an inversion system involving ST, AR, and PP. Thus, the fact that PP occurs frequently in populations in which ST and AR are also frequent is not in opposition to Wallace's hypothesis. However, this has been ascertained only by direct genetic tests of crossing over in the ST/PP heterozygote. Wallace's hypothesis is based on the effect of inversions on crossing over, but it has been shown here that these effects cannot always be predicted from cytological evidence alone. It would seem necessary, therefore, to characterize inversion types by their effect on crossing over in any discussion of their role in a coadapted system.

Of perhaps more general interest is the degree to which crossing over is suppressed in the ST/PP heterozygote. It is clear from the data presented in tables 1 and 2 that a number of crossovers are recovered even in the complex, overlapping inversions in heterozygous condition with ST. A few crossovers are expected on the basis of the mechanism described by Sturtevant and Beadle (1936) in which the only crossover types recovered are those which carry no duplications or deletions. It can be shown, however, that double crossovers are expected among the types recoverable from a relatively long simple inversion such as PP when heterozygous with ST (Sturtevant and Beadle, 1936). These crossovers are possible since they result in the production of normally constituted chromatids. Therefore, the double crossover classes as given in table 1 for regions 1-2, 1-3, and 2-3 were to be expected. The absence of observed crossing over, except for one crossover, might possibly result from the location of markers used with relation to the points of inversion. However, since both *Bl* and *Sc* lie within the inversion, double crossovers should have been recovered. Therefore the nature of the suppression of crossing over between the ST and PP chromosomes must be investigated further. It is clear, however, that it is unwise to draw inferences regarding crossing over in inversion heterozygotes from the cytological configurations of pairing as given by salivary gland chromosomes alone.

An important test of the triad hypothesis results from the presence of the new, simple VF inversion in the San Jacinto Mountain populations. Since there is almost free recombination between VF and ST, a stabilized coadapted system with ST is not predicted by Wallace's hypothesis. Data on the frequencies of this new gene arrangement in the San Jacinto populations which will bear upon the hypothesis are being gathered by Epling (personal communication).

Of the remaining inversions tested it may be said that except for AR, they show as expected an almost complete suppression of crossing over with ST. Thus, according to Wallace's hypothesis and the crossover data, stabilized systems of these inversions with ST are possible.

The data presented here show that crossing over in simple third chromosome inversion heterozygotes of *D. pseudoobscura* may range from being completely suppressed as was the case with ST/PP to being almost normal as shown by the ST/VF heterozygote. The complex, overlapping inversions, however, behave as expected in that they show almost complete crossover suppression. Coupled with these differences in crossover suppression are the interchromosomal effects of these inversions (Levine and Levine 1954) and the variation in crossing over between different strains of the same gene arrangement (Levine and Levine 1955). These phenomena indicate the intricate nature of gene recombination in an inversion system and suggest the need for an evaluation of the role of recombination in the genetics of natural populations.

SUMMARY

Crossing over between the ST gene arrangement of *D. pseudoobscura* and various third chromosome inversions has been analyzed and considered in light of the triad hypothesis of Wallace (1953). It has been shown that an inversion's role in a coadapted system cannot be predicted from cytological evidence alone without confirmatory crossover data. This was demonstrated for the Standard/Pikes Peak heterozygote by the presence of only one double crossover in the total of 6105 flies. The relationship of this finding to the formation of stabilized coadapted gene combinations between the ST and PP arrangements is discussed. Since Pikes Peak is a large simple inversion with respect to Standard, two strand double crossovers are expected. This almost complete lack of crossing over cannot be explained on the basis of any simple mechanism of crossover suppression. In addition, data are presented for the crossing over between ST and a newly discovered simple, third chromosome inversion.

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FLORAL ISOLATION IN PENSTEMON[1]

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In cismontane southern California there occur four species of the section Peltanthera of Penstemon (Scrophulariaceae). These four species are conspicuous in themselves and are all conspicuously different from one another, yet they are so closely related that they are apparently interfertile in all possible directions. While each species is different in its habitat preferences, three of the six possible ecologically sympatric combinations are of common occurence, two may be found fairly frequently, and only one has not been found at all. Despite the relative laxity of the genetic and ecological barriers between these species, there is little tendency to hybridize and no tendency to become swamped evident in any of them. Each of these four species is a geographic representative of a distinct species-group with similar morphological and adaptive characteristics, other members of which meet in other areas over the range of the section to which they belong with the same lack of important hybridization. It is apparent that some additional isolating mechanism is contributing greatly to the prevention of genetic amalgamation of these species; the purpose of the present paper is to inquire into the nature of that isolation.

THE FOUR SPECIES OF PENSTEMON

Penstemon centranthifolius is characterized by bright scarlet flowers about an inch long with narrowly tubular shapes and short, erect, sub-equal limbs. The flowers are borne on lax pedicels in a strict thyrsoid or racemose inflorescence that may occupy half or more of the length of the erect, unbranched stem, and they contrast strongly with the glaucous foliage. This species is ecologically tolerant and widespread in southern and west-central California and northern Baja California.

The other extreme of floral morphology is represented by *Penstemon grinnellii,* whose flower has a short, narrow tube but a broadly and abruptly expanded throat and ample lips. The flower is white suffused with pink or blue in the upper throat and, particularly, on the lower lip, and it has a conspicuously exserted staminode which is strongly bearded. The plant is low, branched, and spreading, the herbage is dark green, and the broadly thyrsoid inflorescence is often rather leafy. This species is an inhabitant of open sites in the montane yellow pine forest, which it frequently shares with *P. centranthifolius;* to the north of the area under consideration it is also found at lower elevations in other communities.

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[1] The following paper represents a portion of a doctoral thesis prepared at the Claremont Graduate School, Claremont, California. The essentials of this paper were presented at the Annual Meeting of the Society for the Study of Evolution in Austin, Texas, on April 8, 1955, under the same title.

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The other two species are intermediate in the shapes of their flowers. *Penstemon spectabilis* has a longer tube than *P. grinnellii,* but its throat is only moderately expanded, its staminode is glabrous, and its flowers are various shades of bright blue or purple. This species is a tall, erect, branched perennial with dark green foliage and an ample thyrsoid inflorescence. It is a pioneer in disturbed sites in the chaparral and coastal sage scrub communities, where it is often accompanied by *P. centranthifolius,* and where it may occasionally meet *P. grinnellii* also.

The fourth species in cismontane southern California is *Penstemon clevelandii,* whose flowers have essentially the same shape as those of *P. spectabilis,* but are significantly smaller and of intense magenta colors. Its staminode is usually short-bearded, its habit is low and spreading, and its inflorescence is moderately ample. This species is typically found in the desert-edge canyons in chaparral and pinyon-juniper woods, where it frequently meets *P. centranthifolius,* occasionally meets *P. spectabilis,* but rarely if ever comes into contact with *P. grinnellii.*

POLLINATING AGENTS

With such diverse flower types, ranging from narrowly tubular to broadly open throats, and such different colors, from scarlet through magenta and blue-purple to nearly white, it seems logical to expect diverse pollinating agents. This expectation is confirmed by field observations.

The large-flowered *Penstemon grinnellii* is well adapted for normal pollination by large carpenter bees of the genus Xylocopa (Apidae) (Merritt 1897, Grant unpubl., Straw 1955a), of which two species (*XX. californica* and *orpifex*) are regularly found attending these flowers. Their stout bodies nearly fill the cavity of the throat of this Penstemon as they feed on nectar hidden in the tube. The bees' short probosces are matched by the short tube length of the flower, and angled grooves along the corners of the lower lips provide strategically placed footrests for the bees also. In entering the throat, pollen is abundantly dusted onto the thick hairs of the upper thorax and first segments of the abdomen of the bee, and transfer to the receptive stigmas in the second stage of development of the protandrous flower is quite inevitable. Because of their size, the carpenter bees are unable to enter the corollas of any of the other three species mentioned here, and in the cismontane region are thus quite exclusively the pollinators of this one Penstemon taxon.

Penstemon spectabilis, the next-largest-flowered species, is visited abundantly throughout its range by wasps of the genus Pseudomasaris (Vespidae). Two species have been collected, of which *Ps. vespoides* is rather common, *Ps. wheeleri* rare. These wasps are unusual in that they provision their nests with pollen rather than animal materials (Hicks 1927). Although they range widely over the western United States, the larger species of this wasp genus appear to be restricted to Penstemon flowers of the size, shape, and blue colors of *P. spectabilis,* and they have been recorded at a number of these other taxa in other areas (Cooper and Bequaert 1950, Cooper 1952, Straw unpubl.). Although they are physically able to enter *Penstemon grinnellii* and feed on its nectar, *Pseudomasaris* species have never been observed to do so; from the other two Penstemons with which we are here concerned they are limited by their size. Thus, the normal pollinator of this Penstemon seems also to be restricted to it within its range.

There is relatively little information available concerning the pollination of *Penstemon clevelandii* on a quantitative basis, but it is visited and pollinated by both solitary bees (Anthophoridae) and hummingbirds.

Each of the aforementioned three insect-pollinated species is visited by a variety of other bees as well, especially small leafcutters (Megachilidae), solitary bees, and bumblebees. For various reasons (size, habits, etc.) which there is not room to discuss in detail here, most of these other bees are considered relatively (but not totally) ineffective pollinators.

The fourth species, *Penstemon centranthifolius,* is a "typical" hummingbird flower, and is visited by both Anna and Costa hummingbirds. The regular outline of the limb surrounding the orifice furnishes a target for the bill, and the narrow tube serves as a guide toward the nectar, as well as to direct the bill past the anthers and stigma to effect pollination. The very short limb and small diameter of the throat in this species prevent most insects from entering, although some of the very small leafcutters, e.g., *Hoplitis producta,* sometimes enter the corolla and take pollen. On the whole, however, insect visits to *P. centranthifolius* have been observed only infrequently.

Although it seems apparent that each of these four species of Penstemon has its own normal pollinators, carpenter bees, wasps, solitary bees, and hummingbirds, there still remains the crucial problem as to how exclusive these pollinators are. In nature the most common species-pairs found together include *P. centranthifolius* as one of their members; that is to say, a hummingbird-pollinated and an insect-pollinated species are found together more frequently than are two insect-pollinated species. Because of the narrow shape and small orifice diameter of *P. centranthifolius* few insect visitors to the other species are able to enter it, and of those that are able, few have been observed to do so. It has been determined from spectrophotometric analysis of the color of *P. centranthifolius* that almost the entire reflectance spectrum of this corolla lies in the red bands (Straw 1955a), beyond the limits of visibility of such bees as have been studied in this regard (von Frisch 1914, Bertholf 1931, *et. al.*). Assuming an essential analogy to human vision (Griffin 1953), this means that to the bee this corolla must be nearly without color, that is, black or some very dull shade. Such colors do not seem regularly to attract the day-active bees. Insect pollination of this scarlet-flowered species thus is strongly inhibited.

Of the insect-pollinated species that might be found together, the normal carpenter bee pollinators of *P. grinnellii* and the masarid wasp pollinators of *P. spectabilis* are limited to their own preferred species by their larger sizes and, in the case of the wasps, by their essential flower constancy. Although *P. grinnellii* grows well within the altitudinal range of *Pseudomasaris,* the wasp has never been observed at that species. The preference of the wasp for corollas of the blue color and moderate size exemplified by *P. spectabilis* seems to be exclusive.

Concerning the bees that visit *Penstemon clevelandii* we have little information beyond the fact that none of the species found is restricted to this flower, but may visit frequently the flowers of other species of Penstemon and other genera as well. This is particularly true of the many megachilid bees, such as species of Osmia and Anthocopa, that are widely distributed throughout this region. We cannot conclude from this that these bees are individually promiscuous, however. On the contrary, they may be very constant to a single kind of flower over any given period of time, just as the honeybee and many bumblebees are known to be (Grant 1950). Michener (1953) has presented evidence that one species of leafcutter (*Megachile brevis*) is quite constant to a single species of flower while collecting pollen, but may be promiscuous when feeding on nectar only. At least one leafcutter, *Ashmeadiella australis,* is restricted to Penstemon (Michener 1939), and in southern California, apparently to *P. grinnellii;* its habits are apparently very constant.

This leaves for consideration the hummingbirds, of which it is well known that, although they may be the almost exclusive pollinators of *P. centranthifolius,* they are by no means restricted either to this species or to this genus of plants. Close observations of the birds in mixed colonies of Penstemon has lead to the belief that the hummingbirds are individually relatively constant to single feeding locales and, over the minimum period of a single feeding flight, to a single species of flower. This conclusion is not out of line with other detailed studies of the habits of hummingbirds (e.g., Bené 1942, Wagner 1946), despite the birds' reputations for promiscuity. Hummingbirds have been seen to cross from one Penstemon species to another on consecutive feeding flights, however, and by such acts might cause hybridizations. Several factors may tend to minimize the chances of illegitimate pollinations under these circumstances. On the one hand, the birds are seen frequently to clean their bills during the pauses between flights, which, if it does not eliminate, must at least greatly reduce the amount of pollen that may be carried from one species to another over the intervening period. On the other hand, while it is almost impossible for a hummingbird to probe a flower of *P. centranthifolius* in the proper stage without obtaining a considerable amount of pollen on its bill or placing pollen on the stigma, in the larger, insect-pollinated species the bill may touch the essential organs only accidentally. In *P. grinnellii* the birds have been observed habitually to enter the corollas from so oblique an angle that pollen might be placed on the stigma only with extreme rarity.

ISOLATING MECHANISMS

It seems evident that this whole complex of ethological and mechanical characteristics of the flowers and their pollinators has helped to keep the four species of Penstemon mentioned here quite discrete from one another, and has permitted the sympatric occurrence of species between which genetic, ecological, temporal, and other barriers are quite inefficient. Since the mechanism depends on the habits and discriminatory abilities of the animal pollinators as well as on a number of purely mechanical factors, it is thought best to refer to this system as "floral isolation" (Grant 1949), in a rank coordinate with the other types of isolating mechanisms generally distinguished in biological systems (e.g., Stebbins 1950, Chapter 6; Riley 1952).

In some situations floral isolation may be nearly 100 per cent effective, as, apparently, in a case described in Delphinium by Epling (1947, as discussed by Grant 1949: 90 ff.). Complete dependence upon one specific pollinator may, however, result in the infertility of a colony if that normal pollinator should for some reason fail to appear, and in the long run might lead to the extinction of the species as a result of overspecialization. In these species of Penstemon floral isolation is not so highly developed as to run this risk, since insects other than their normal pollinators may, with a frequency dependent largely upon their size, sometimes pollinate them. These species are also all perennials, to which a reduced seed set in any one year would not be so destructive an event as if they were annuals.

Successful hybridization, furthermore, is not unknown. One hybrid, *Penstemon centranthifolius* × *spectabilis* (= *P.* × *parishii*), is rather common in the areas where its parents occur together, and is a distinctive and easily recognizable entity. A second hybrid, *P. centranthifolius* × *grinnellii* (= *P.* × *dubius*), has been found twice. The appearances of such characters as bearded staminodes in colonies of *PP. spectabilis* and *centranthifolius* and modifications in the corolla proportions in *PP. centranthifolius* and *clevelandii* seem to indicate further gene flow between other species. The characters of the plants in these last cases are so confused that it is at present impossible confidently to defend the hypothesis of introgression which is, nevertheless, tentatively advanced.

We must not interpret this evidence of gene exchange as detrimental to the hypothesis of floral isolation, however. In the species where no clear-cut cases of hybridization are known, the evidences for introgression are of a questionable nature also, while of the two natural hybrids that are known, one has been found only twice in an area where its parents overlap that has been carefully explored by botanists for many years, and the other is deserving of special consideration.

The case of the rather common occurrence of *Penstemon centranthifolius* × *spectabilis* is particularly interesting. About ten hybrid individuals have been seen in the field during the past two years, and in every case they were in very small mixed colonies of the parents (usually fewer than thirty individuals of both kinds) rather than in the more extensive mixed colonies that may also be found. It is suggested that the small numbers of plants of any one species available for feeding upon in these small aggregations may cause the normally constant pollinators, here probably hummingbirds, to break their constancy and seek food in the flowers of the other species, thereby making illegitimate pollinations. Colony size appears to affect the constancy of insect pollinators in a similar manner.

The colonies in which hybrids are found are somewhat unusual also, in that in no case seen by me has it been possible to identify in the field or to detect by special means what might be called a hybrid swarm. The colonies observed have regularly contained the two parents and one or two obvious hybrids, but none of the intergrading segregates or backcrosses one would expect to find. If either segregates or backcrosses have been present their morphology has been such that they could not reliably be distinguished from the parents or the hybrids, and the plants all fall easily into the three classes. The hybrid is highly fertile (average of ten plants, 65.7 per cent good pollen, as measured by stainability in aniline blue and lacto-phenol) and produces some viable seed. Floral isolation, however, discriminates strongly against these individuals, and in the field they are relatively little visited by the insects and hummingbirds that are attracted to their parents in great numbers. Where the hybrids are visited and backcrossed, this stringent selection appears quickly to draw the hybrid types into conformity with one of the parents. This situation is thus considered to be another example of the mechanism described by Grant (1952) in Aquilegia for the selection toward the parental types in a hybrid swarm. Its only evident result seems to be a rather large variability pattern in *P. spectabilis*. A further mechanism, for the production of new species through hybridization and selective pollination, is described elsewhere (Straw 1955b).

SUMMARY

Four species of Penstemon section Peltanthera in cismontane southern California are very different in appearance and occur in several sympatric combinations. Despite the fact that they are largely interfertile, hybridization is relatively rare. Field studies have shown that each of the species has its own normal range of pollinators, and it is concluded that floral isolation adequately restricts the hybridization that might lead to swamping or merging of the species.

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"NATURAL IMPLANTATION" OF A LETHAL MUTATION IN *DROSOPHILA MELANOGASTER*

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Many lethal mutations are characterized by different reactions of the various organ systems. Certain parts may become entirely abnormal, whereas others are only slightly affected. The great variation in developmental potencies of the separate parts can be studied by isolating them from their surroundings in the lethal individual. This has been done for a number of lethal genotypes, mainly by Hadorn and his collaborators (for detailed references see Hadorn 1950, 1951). Observations were made on the capacity for autonomous differentiation of primordia from lethal larvae when transplanted into genetically normal hosts. Thereby two major categories of effects can be distinguished. Certain organ systems do not develop beyond the stage they would have reached in the lethal individual; cell factors that are intrinsic or at least determined prior to transplantation thus seem responsible for the inhibited capacity of differentiation in these cases. Other structures, however, seem to develop normally and can sometimes even be shown to function normally.

This elegant technique has produced some very interesting information, but its application offers certain restrictions. Transplantation is only feasible in late larval stages and hence mutations which exert their killing effects earlier in development cannot be studied by this method. It is also practically impossible to study whole organ-systems for this would involve gross transplantations which could hardly be made because of their complexity. Furthermore, as Hadorn (1951) points out, failure of development after transplantation may lead us to the erroneous conclusion of direct damage by some intrinsic factors. Exchange of transplants is restricted to the larval stages only, and therefore, transference to normal surroundings may have come too late to undo irreparable damage to certain systems, which had already been exposed for too long a time to the unfavorable extrinsic influences of the lethal milieu.

More information as to the latter problem has been obtained by a very different approach. Demerec (1934), Ephrussi (1934), and Stern (1935) used stocks which increased the frequency of somatic segregation, crossing-over,

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elimination or non-disjunction, in order to obtain small patches of tissue homo- or hemizygous for both visible mutations and recessive lethal deficiencies. In these cases the lethal-bearing cells were thus surrounded by normal tissue from the onset of development. Under such conditions some lethals were viable, while others caused inviability even in single cells that were embedded in a normal blastema. Therefore one may conclude that certain lethals are characterized by a cell-intrinsic cell-lethality.

Another possibility for studying the effect of lethal gene action would be by an analysis of sex-linked recessive lethals in gynanders. This method relies on the fact that an egg one of whose X chromosomes gets lost or undergoes a gross deletion during an early cleavage stage gives rise to a gynander or mosaic individual. If the maternal X-chromosome in addition to visible markers carries a recessive lethal and the paternal X-chromosome is lost during the first cleavage it would be possible, theoretically, to obtain flies half of whose bodies are hemizygous for the lethal in question. Loss of the paternal X during a subsequent division would result in smaller hemizygous areas. Supposing now, that this lethal is not a cell-lethal, the problem arises, how large an area of hemizygous lethal-bearing tissues could be borne with impunity. The use of gynanders as a means for studying sexual differentiation in *Drosophila* was originally suggested by Muller (personal communication). Sturtevant (1920) has used gynanders to show that the eye color mutation, vermilion, undergoes non-autonomous differentiation, unlike most of the other eye color mutants in the fruit fly. Other workers (Patterson and Stone 1938, Stern and Hannah 1950, Bonnier 1950, Novitski 1951, Hannah 1953, and Stern 1954) have also utilized this technique to determine the degree of autonomy exhibited during the growth, differentiation, and functioning of various other imaginal tissues in *Drosophila*.

In this study we used the mutation lethal(1) melanoma-like (*l*ml,1-1.0) (Oster, 1952). Males carrying *l*ml die in the third larval instar with many black melanotic inclusions uniformly distributed throughout the body. We thereby hoped to obtain some information on the origin and distribution of these melanotic "tumors" in the mosaic individuals. Similar studies on a number of other sex-linked lethals are in progress now.

MATERIALS AND METHODS

Previously Muller (1940, 1954) had shown that irradiation increases the frequency of ring-X-chromosome loss, such as the one designated X^{c2}, and the subsequent formation of gynanders. For this reason males containing X^{c2} were irradiated in two series of experiments with 4000 to 5000 r and mated to females of the composition: $\dfrac{y\ l\text{ml}\ w\ \text{In49 f.}\ Y^{s}}{sc^{S1}\ \text{B InS}}$. In the second series the females were aged for ten or more days, as Brown and Hannah (1952) had shown that the ring-X-chromosome is eliminated with a higher frequency when introduced into eggs derived from aged females. After three days the male parents were discarded and the females were transferred to fresh culture bottles in which they were allowed to lay eggs for an additional five days.

Three F_1 classes of flies were obtained:

$$\underset{\text{normal (+) ♀}}{\frac{\text{y}\ \mathit{l}\text{ml w In49 f. Y}^{s}}{\text{X}^{c2}}} \qquad \underset{\text{Bar (B) ♀}}{\frac{\text{sc}^{S1}\ \text{B InS}}{\text{sc X}^{c2}}} \qquad \underset{\text{scute Bar (sc B) ♂}}{\frac{\text{sc}^{S1}\ \text{B InS}}{\text{Y}}}$$

The fourth class of flies, that is, males of the composition y *l*ml w In49 f.Y^s, did not survive because of the presence of *l*ml.

Yellow (y), white (w), and forked (f) were used as visible markers for the detection of hemizygous areas in the normal class of F_1 females. In the Bar (B) class of females, scute (sc) and Bar (B), served the same purpose. The presence of inversions (designated sc^{S1} and InS) served to suppress somatic crossing-over in the F_1 offspring.

RESULTS

The results are summarized in the accompanying tables.

TABLE 1

The frequency of gynanders and mosaics in the offspring from the cross:

$$P_1\ \frac{\text{y}\ \mathit{l}\text{ml w In49 f. Y}^{s}}{\text{sc}^{S1}\ \text{B InS}}\ ♀ \times \frac{\text{X}^{c2}}{\leftharpoondown}\ ♂$$

F_1

	+♀	B♀	sc B♂	Gynanders and/or Mosaic Individuals	
				+♀	B♀
Series I	1867	1731	724	No. 1: ½ of abdomen yellow; external genitalia male-like; small melanotic area near genitalia.	No. 1: 1 eye hemizygous Bar; whole head scute.
					No. 2: 1 eye hemizygous Bar; whole head scute; 1 sex comb.
					No. 3: ½ of thorax scute; 1 wing shorter (i.e., male-like).
					No. 4: ½ of thorax scute; 1 wing shorter (i.e., male-like).
				$^1/_{1868}$ = 0.053%	$^4/_{1735}$ = 0.23%

TABLE 2

				Gynanders and/or Mosaic Individuals	
	+♀	B♀	sc B♂	+♀	B♀
Series II	1129	953	1122	No. 1: less than 1/10 of 1 eye white.	No. 1: external genitalia male-like.
				No. 2: 1/10 of 1 eye white.	No. 2: orbital, ocellar, vertical, postvertical and scutellar bristles missing.
				No. 3: 2/5 of 1 eye white.	No. 3: 1/2 of thorax scute.
				No. 4: 1/5 of 1 eye white.	No. 4: 1/2 of scutellum scute; 1 wing shorter (i.e., male-like).
				No. 5: 2/5 of 1 eye white.	No. 5: sternite bristles on 1/2 of abdomen reduced in size; 1 wing shorter (i.e., male-like).
				No. 6: 7/8 of 1 eye white.	No. 6: 1/2 of abdominal tergite pigmentation male-like; 1 wing shorter (i.e., male-like).
				No. 7: 1 eye all white with the exception of a few red facets; 1/2 of head yellow and forked.	No. 7: 1 eye hemizygous Bar; 1/2 of head scute.
					No. 8: 1 eye hemizygous Bar; thoracic bristles strongly reduced in number; sternite bristles reduced in size and number; 1 sex comb; 1 wing shorter (i.e., male-like).
				7/1136 = 0.62%	8/961 = 0.83%

CONCLUSIONS AND DISCUSSION

The observation of various mosaic flies among the "normal" (y *l*ml w In49 f.Y^{s}/X^{c2}) class of females shows that individuals with patches presumably hemizygous for *l*ml can survive to the imaginal stage. Thus, the mutant, *l*ml, at least in the hypodermal cells, does not act as a "cell-lethal." Gynanders with sizeable areas of hemizygous tissue were, however, completely missing among the "normal" females. A comparison of the size of the mosaic parts in the two classes of females shows that the hemizygous areas were much smaller in the "normal" than in the Bar-eyed class of females. It seems as if in individuals with sizeable areas hemizygous for *l*ml, the deleterious effects of this gene prevent the developing animal from reaching maturity. Another way of explaining the limited size of patches hemizygous for *l*ml might be to assume that there is a retarded growth in mutant tissues, as compared with the growth rate of normal cells. Observations on the developmental rate of ordinary *l*ml males, however, do not support this hypothesis. As the size of the mosaic parts can be correlated with the time of elimination of the ring-chromosome it is clear that *l*ml exerts its killing effects mainly in those cases where the paternal X-chromosome is lost at a relatively early cleavage. On the other hand, those individuals which have lost the ring during a late cleavage (that is, those having small hemizygous areas) are capable of surviving to adulthood.

Considering the mosaics in the class of normal females in Series II it is apparent that in the vast majority of these cases only the marker white (*w*) shows up. Some of these mosaics may possibly be due to deficiencies for the white locus, caused by the heavy irradiation of the paternal X-chromosome. They therefore may still contain the normal allele of *l*ml. But the observation of a mosaic fly showing all markers, namely yellow, white, and forked (no. 7 in Series II), and the fact that *l*ml is closely linked to white, both support the conclusion that lethal(1) melanoma-like does not act as a cell-lethal.

The unusually high frequency of individuals with small mosaic areas warrants a further consideration in view of the previous findings of Battacharya (working under Muller's direction), who failed to detect any increase in the number of small patches of white facets in the eyes of the female offspring of irradiated ring-X males (Muller 1940, 1954, Battacharya, 1950). In the first series, our results were essentially similar, that is, the mosaic individuals which we found contained large hemizygous areas. However, in our second series, in which we had used aged females, we found many cases of individuals bearing small mosaic areas both among the normal and Bar-eyed female offspring. In all probability they were caused by the aging of the females, mediated perhaps by some unknown cytoplasmic factor(s), and not due to the radiation. This is in line with the results of Brown and Hannah (1952), who reported a similar increase in the number of small mosaic areas in the offspring of crosses of non-irradiated ring-X males mated to aged females.

This incidental finding which is under further study can now be made use of in studies similar to the one described above for determining whether a particular mutant is a cell-lethal in the same cross as that used for studying the mutant's behavior in gynanders. That is, irradiation of the ring-X would result in gynanders if flies bearing sizeable areas of the particular lethal being studied are viable, while the appearance of small mosaic areas produced by the aging of the female parent would indicate that the lethal in question is not a cell-lethal.

SUMMARY

A method is described for the production of mosaic flies with tissues hemizygous for sex-linked recessive lethals. Our investigation deals with

the mutant, lethal(1) melanoma-like (*l*ml, 1-1.0). Individuals with patches hemizygous for this sex-linked lethal are viable, but it was observed that these areas were much smaller than mosaic parts in gynanders of the control class. It therefore appears that *l*ml does not act as a cell-lethal but that its deleterious effects prevent the animal from reaching the imaginal stage if sizeable areas are under the influence of this gene.

ACKNOWLEDGMENTS

The authors wish to express their appreciation to Professor H. J. Muller and Dr. C. Auerbach for many stimulating discussions during the course of the work and to Professor C. H. Waddington for kindly providing the facilities with which the latter part of this study was carried out.

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LETTERS TO THE EDITORS

Correspondents alone are responsible for statements and opinions expressed. Letters are dated when received in the editorial office.

COMMENTS ON DR. WHITE'S ARTICLE ON TISSUE CULTURE

Dear Sir,

I have read with much interest Dr. Philip White's stimulating article on "Cellular Biology and Tissue Culture."

Dr. White states that Tissue Culture has not entirely fulfilled the extravagantly high hopes with which it was originally regarded, and I think that there are two main reasons for this. In some ways the method was born before its time. When it was found that living cells could be cultivated outside the body, it seemed at first that we had been provided with perfect material for studying the physiology of growth. In practice, however, it proved extremely difficult to obtain accurate quantitative information about the metabolism of tissue cultures, because the microchemical techniques required for such delicate work have only become available during the past few years. To some extent this technical limitation also applied to the study of individual living cells in vitro, but the recent development of ultra-violet and interference microscopy, for which the thin, well spread cells of tissue cultures are ideally suited, has opened a new approach to the quantitative investigation of many aspects of cellular physiology.

In the second place, for many years there was an unfortunate tendency to elevate Tissue Culture to the status of a separate branch of biology, so that its true function as merely "a versatile tool" (to quote Dr. White) was not always appreciated. I myself have often been approached by people who wished to learn Tissue Culture without having any definite problem in view and who were obviously quite unaware of either the possibilities or the limitations of the technique. In some laboratories this woolly attitude of mind has meant that Tissue Culture has been asked the wrong questions or, indeed, no questions at all; a mass of valueless observations have resulted, while many important problems which the technique might have solved have been neglected.

As Dr. White points out, in the past certain tissue culturists "permitted themselves to be somewhat more fascinated by the techniques than by their proper objectives." The perfecting of a Tissue Culture method is a will-o'-the-wisp which can be pursued indefinitely with much personal enjoyment but little scientific advantage, and in general it is more profitable to develop the simplest possible technique that will serve the investigator's purpose and then concentrate on the research itself. At the present time there tends to be a cleavage between the 'simple' and the 'complex' schools of Tissue Culture, but in fact the complexity of the procedure should depend entirely on the nature of the problem to be studied. On the one hand we should not, out of technical snobbery, reject a method which would be entirely suitable for a certain investigation, just because it happens to be simple and old fashioned, but on the other hand we should recognise the fact that some types of research may demand an elaborate and costly organisation. The important thing is not to waste time and money on a complicated technique where a simple one will do equally well (and sometimes much better).

During the past decade, Tissue Culture has been contributing to biological and medical research with ever increasing success. The technique is being used in a much more realistic and fruitful way than formerly, and as Dr. White has indicated, it is now being applied to many widely different problems which cannot be satisfactorily studied in the intact organism. This renaissance dates from the Hershey Conference in 1946, and owes much to the imaginative and enterprising officers of the American Tissue Culture Association.

Yours etc.,

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Sept. 1, 1955

CROSSING OVER WITHIN INVERTED REGIONS IN CHIRONOMUS.

In those animals in which inversion heterozygosity is common, and in which there is crossing over in the male, it has been supposed that chiasmata must nearly always be confined to those regions of the chromosome in which there are no inversions (Philip, 1942; White, 1954). Though the abnormal chromatids which result from crossing over in inversion-loops are known in some cases to be eliminated in the female (Sturtevant and Beadle, 1936; Carson, 1946), the same mechanism cannot exist in the male.

Chiasmata can be seen in the testes of *Chironomus thummi*, *C. dorsalis*, *C. annularius* and *C. tentans.* Crossing over is known definitely to take place in both sexes in *C. tentans* (Beermann, 1952), and it follows that there may be crossing over between certain inverted sequences which would lead to the production of abnormal chromosomes. In the left arm of chromosome 2 in this species (Beermann, 1955) there are several different, mutually exclusive, sequences. Crossing over in individuals heterozygous for two of these sequences will lead to the formation of a chromosome, not dicentric, with a short duplication and deficiency. These, presumably, cannot be eliminated in oogenesis as there will be no anaphase bridge, and most of the abnormal chromosomes must eventually be eliminated by selection. Nevertheless, I have found one larva among 1600 carrying such a chromosome. It is apparent then that crossing over does occur between some arrangements, and that at least occasionally abnormal chromosomes are produced which are not lethal.

No evidence of crossing over within the inverted region of an inversion heterozygote has been reported in male Chironomidae, though a study of spermatogenesis in *C. dorsalis* has been made in which no evidence of bridges and fragments was seen (Philip, 1942). I have examined larvae of the same species from a population in which the frequency of one of the inversion heterozygotes approached 50%. A number of suitable squash-preparations was made of the testes of early prepupae. One of these preparations was of the testis of a prepupa heterozygous (judged from its giant chromosomes) for three inversions, one in each of the two longest chromosomes (1 and 2) and one in the shortest (4). Seven good examples of bridges and fragments were seen; the fragments were of two types, one long (Fig. 1) and the other short (Fig. 2). Chromosome 4 is easily identified, so that the fragments must have been the result of crossing over within each of the remaining inversions. In a second preparation in which there was only one of these inversions (chromosome 1), the fragments were short, so that the longer fragment in the first preparation must correspond to the inversion in chromosome 2. No quantitative data on the frequency with which bridges and fragments are formed are yet available, and may be difficult to get as their presence, or absence, in a given preparation may depend on the age of the testis.

In all populations of *C. dorsalis* so far studied inversion heterozygotes have been found at appreciable frequencies, and consequently the deleteri-

FIG. 1 FIG. 2

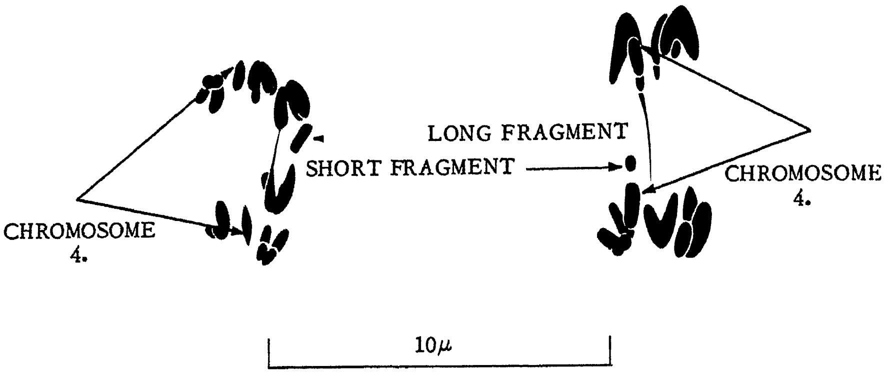
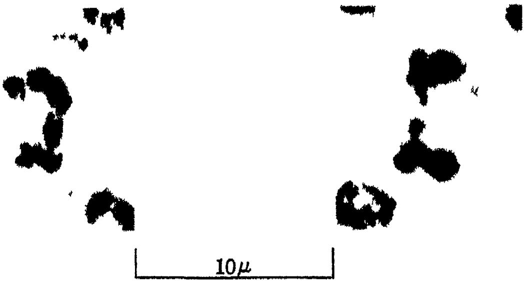


FIG. 1 FIG. 2



CHIRONOMUS DORSALIS—BRIDGES AND FRAGMENTS IN TESTIS OF LARVA HETEROZYGOUS FOR INVERSIONS IN THREE CHROMOSOMES

ous effects of crossing over for the population cannot be mitigated by the fact that inversions are rare. Furthermore, the number of heterozygotes in which crossing over will produce abnormal chromosomes without the formation of a bridge at meiotic anaphase is also quite high in some populations. Unless crossing over in inversion heterozygotes is very rare the selective values of the various combinations of chromosomes arrangements must be high enough to outweigh this disadvantage. Further details will be published elsewhere.

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COMPARISON OF X-RAY-INDUCED MUTATION RATES IN DROSOPHILA AND MICE

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COMPARISON OF X-RAY-INDUCED MUTATION RATES IN DROSOPHILA AND MICE[1]

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Until recent years, estimates of genetic hazards of ionizing radiation in man were based primarily on information obtained from Drosophila. Our investigations (Russell, 1951) on radiation-induced mutation rates in mice, and a comparison of the results with as nearly similar data as were available for Drosophila, showed a higher mean rate in the mouse, and led to the conclusion "that estimates of human hazards based on Drosophila mutation rates may be too low." It was recognized that the Drosophila data that were available were not ideally suited for comparison with the mouse data. Thus, the mouse mutation rates were determined for irradiated spermatogonia, utilizing seven specific autosomal loci, and there was no information on specific-loci rates for irradiated spermatogonia in Drosophila, although there was information on specific-loci rates for irradiated spermatozoa. We considered this a serious lack and we, therefore, suggested and supported the study made in our laboratory by Alexander (1954) which was designed specifically to provide Drosophila data more suitable for comparison with the mouse. In this investigation, the mutation rates were determined: (a) at specific loci, (b) on autosomes, (c) for irradiated spermatogonia as well as spermatozoa, (d) with essentially the same genetic technique and method of scoring the mutations as had been used on the mice, and (e) with the same X-ray machine and method of dosimetry as that used in the mouse experiment. Alexander mentions the mouse mutation rate in her publication, but she does not make a detailed comparison of her data with the mouse data, it having been decided to postpone this until additional mouse data were published. It now appears important to make a detailed comparison without further delay. One reason for this is the suddenly increased interest in genetic hazards of radiation. Another reason is the recent paper by Ives (1954) in which he discusses radiation-induced mutation rates in Drosophila and mice, using our mouse data, and Drosophila data of Alexander (1954)[2] and himself. He concludes "that for the present the radiation-induced mutation rate per r per locus appears to be similar in flies and mice." Thus Ives disagrees with the conclusion I stated in 1951, that comparison of the mouse data with similar data from Drosophila shows a higher induced mutation rate in the mouse. Since I believe the new Drosophila data of both Alexander and Ives support my original contention, and since this question has a bearing on the important problem of estimating genetic hazards of radiation in man, it seems necessary to discuss the species comparison at some length and point out what I consider to be major errors in Ives' argument.

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[1] Work performed under Contract No. W-7405-Eng-26 for the U. S. Atomic Energy Commission.

[2] Ives' paper does not include a reference to this particular article, which perhaps had not appeared when Ives submitted his paper for publication. The only publication of Alexander's that he cites is an earlier abstract. However, it is clear from Ives' treatment of Alexander's data that he had access to a much more extended report of her work than was given in her abstract.

Perhaps the most serious fault of Ives' paper is that nowhere does it contain a discussion of Alexander's spermatogonia data. In order to ensure that these data came from irradiated spermatogonia, Alexander treated the flies in early larval stage. Although this involved painstaking work, it was undertaken because, as has already been pointed out, it was considered important to have Drosophila data comparable to the mouse data which, because of the importance of the spermatogonial stage in human hazards, had been obtained from irradiated spermatogonia. Yet Ives, despite the fact that his paper is devoted to a consideration of the species comparison, completely ignores the Drosophila spermatogonia results and discusses only the data on spermatozoa which Alexander obtained for comparison with her spermatogonia results and for future comparison with mouse spermatozoa results when these become extensive enough. Throughout his paper Ives uses only the Drosophila spermatozoa mutation rates for comparison with the mouse rate in spermatogonia, which, incidentally, he wrongly calls "immature sperm." Since he mentions that I pointed out the complication in making such a comparison, it is clear that he should have been aware of the desirability of having spermatogonia data in Drosophila. It is, therefore, especially hard to understand why he does not discuss Alexander's spermatogonia rate and why he even fails to mention that she worked on spermatogonia as well as on spermatozoa. Even if nothing were known about the quantitative and qualitative difference between mutants recovered from spermatozoa and spermatogonia, it would seem to be good judgment to use the same cell stage in making comparisons between species. Actually, since the work of Hertwig (reviewed by Russell, 1954) much has been known about the difference in results obtained from irradiated spermatozoa and spermatogonia in the mouse. Thus, there is a virtual absence of dominant lethals and translocations in offspring obtained from gametes that were irradiated as spermatogonia with doses that cause a high incidence of these aberrations in irradiated spermatozoa. There are, therefore, actual experimental data showing that, whenever possible, it is, indeed, desirable to use the same cell stage in making comparisons.

Ives' failure to appreciate the qualitative difference in results obtained from irradiated spermatozoa and irradiated spermatogonia leads him into making comparisons that are not valid. For example, he says sterility and inviability of the mutants are "apparently much higher in Drosophila than in mice." Since he is comparing mutants from irradiated spermatozoa in Drosophila with those from irradiated spermatogonia in mice, it cannot be claimed that the difference in behavior of the mutants is a species difference. There is evidence that indicates that a large part, if not all, of the difference may be a result of difference in the cell stage irradiated. For example, it has long been known (see review by Russell, 1954) that sterility, in general, is common in the offspring resulting from irradiated spermatozoa, but not in the progeny from irradiated spermatogonia. In her paper, Alexander does not comment on the viability and fertility of presumed mutants in her spermatogonia experiment, but it is apparent, from a private communication, that, in these respects, her Drosophila spermatogonia data are much closer to the mouse spermatogonia data than are Drosophila spermatozoa data.

Ives cites a "few cases" in his work where apparent mutations are not inherited. He attributes these to "delayed mutations" which involved only the soma, and, he states that, because of the difference in the stage of development at which separation of the germ line occurs in Drosophila and mice, mutation rates will be differentially biased if these cases are scored only when they are heritable. Regardless of whether Ives' interpretation that these are delayed mutations is correct or not, he again makes the error of comparing Drosophila spermatozoa and mouse spermatogonia. Since no non-heritable cases were found in the entire sample of mutations from irradiated mouse spermatogonia, it can safely be concluded that if there are any heritable induced mutations that are delayed in occurrence until embryonic stages, they must be rare and cannot contribute more than an insignificant amount to the observed mouse mutation rate.

In spite of the difficulty of comparing mutation rate in spermatogonia of one species with that in spermatozoa of another species, it is sometimes necessary to make such comparisons. This was the case in my 1951 report on mouse mutations since, as has already been mentioned, no data were available at that time on specific-loci mutation rates in Drosophila spermatogonia. Since, even now, Drosophila data of this kind are not extensive, it is still of some interest to compare the mouse spermatogonia mutation rate with the Drosophila spermatozoa rate. However, the comparison must be made as it was in my 1951 report, namely, with due regard to the difference between the cell stages. This is not the way in which Ives makes the species comparison. In the first place, as will be discussed later, he makes no allowance for a quantitative difference in mutation rate between the cell stages. In the second place, his attributing the frequent inviability and sterility of mutants from irradiated Drosophila spermatozoa to the peculiarity of the species, rather than to the cell stage irradiated, leads him to make the remarkable specification that the species comparison should be based only on "F_1 results." (His other specification that only autosomal loci be used, had already been met in Alexander's experiment.) What Ives calls "F_1 results" may be defined as the total of all those individuals which, by phenotypic criteria, could be mutants, regardless of whether or not they actually test as such. This seems to me to be entirely the wrong criterion to use for the species comparison. Ives qualifies his criterion to some extent by stating "it is important to attempt the genetic identification of the F_1 mutants in order to determine the accuracy of the observer's classification of the F_1." However, as will be shown, he does not apply this qualification consistently.

The illogicality of Ives' position can be shown in the following manner. If an attempt has been made to test all the presumed mutants genetically, they can then be divided into three main groups: (a) those tested and found to be mutations at the specific loci, (b) those tested and found not to be mutations at the specific loci, i.e., phenocopies and mimics, and (c) those which died before testing or were found to be sterile. It is apparent that a maximum estimate of mutation rate is given by (a) + (c). A better estimate would be obtained if any decision could be made as to what portion of category (c) could be attributed to specific-loci mutations. When this cannot be done, comparison of point mutation rates between Drosophila and mice, or between different sets of Drosophila data, are probably best made on the basis of category (a), rather than on (a) + (c). This will be discussed later. In any case, it would seem clear that category (b) should not be included in estimates of the mutation rate at the specific loci. Yet what Ives is proposing is that category (b) be included and that the mutation rate be estimated from (a) + (b) + (c). It is precisely in this way that he calculates his estimate of the mutation rate from Alexander's data. This is not a small matter. In Alexander's data, category (b) is the largest of the three. Thus, Ives' tabulation of mutations in Alexander's data includes three categories, the largest of which Alexander had proved genetically *not* to be mutations at the specific loci.

What Ives apparently does with his own data is to include all three categories in calculating the mutation rate, but only after some investigation as to the size that (b) would have if no qualifications were applied to taking "F_1 results." As a result of this investigation, he concludes that roughoid-like variants prove to fall in class (b) so frequently that taking only "F_1 results" would lead to major error. Instead of recognizing this as an indication that he should discard the method of taking "F_1 results," he then decides to exclude all of his data on the roughoid locus, although in an earlier paper (Ives, Levine and Yost, 1954) he had not done this. (In the earlier paper, Ives gives what seem clearly to be the same data, but the total number of "res-like mutants" for the 3000-r X-ray dose is listed as 68 instead of the 39 given in the later publication. Thus Ives, himself, demonstrates the unreliability of using "F_1 results.") Peach-type variants also give some trouble, but Ives thinks that "with practice" one can distinguish the true peach-locus mutations by their phenotype. By such means, Ives reduces the size of category (b) in the tabulation of his own "F_1 results." Perhaps he has reduced this category to zero. Since he states that he has tested all the presumed mutants in his work, he should know whether or not it is zero. In any case, if category (b) is not large in his data, it is not surprising that the mutation rate that he obtains from his own results is considerably lower than the spurious rate he calculates from Alexander's data, where he is including all of the large group of variants that were proved not to be mutants. It is in category (b) that the results of different investigators will tend to vary, depending on their personal evaluation of which cases are doubtful enough to require testing. Thus, the difference between the "F_1 results" of Alexander and Ives indicates not a difference in mutation rates, but simply that Alexander was either more cautious or less practiced than Ives.

The fact that "F_1 results" do not form a reliable basis on which to make comparisons is illustrated by material already published that makes it possible to compare, both on the basis of tested mutations and "F_1 results," different sets of data from the same species, for the same loci and for approximately the same cell stages irradiated. Thus, if Alexander's data are compared with Patterson's (quoted by Alexander) for the seven loci common to both sets of data, the mutation rates based on tested mutations are 5.6×10^{-8} and 3.4×10^{-8}, respectively, while "mutation rates" based on "F_1 results" would be 41.3×10^{-8} and 8.74×10^{-8}, respectively. Similar comparisons between Alexander's and Ives' results cannot be made accurately since it is not possible from the information provided by Ives to determine what proportion of his 39 "mutant F_1" would be comparable to Alexander's tested mutations. However, from the general statement that "in most series half or more of the *res*-type F_1 mutants have failed in retests because of sterility or inviability" one may conclude that a mutation rate based on tested mutations would be something less than half of 14×10^{-8}, and, therefore, presumably in good agreement with Alexander's 6.3×10^{-8} for the same seven loci. A similar comparison based on "F_1 results" instead of tested mutations gives 14.0×10^{-8} for Ives and 24.4×10^{-8} for Alexander. It is apparent that in mutational data that are expected to be in agreement, close agreemant has been found when the results are compared on the basis of tested mutations, but in these same data there can be wide discrepancy in "F_1 results."

Since "F_1 results" are demonstrably unreliable for comparisons made within Drosophila, and on data obtained from the same loci and from irradiation of the same cell stage, it is apparent that, on this account alone, they should not be chosen as the basis for comparisons between different species and between results from irradiation of different cell stages. Furthermore, for comparisons of this sort, "F_1 results" are likely to be even more unreliable. Thus, the proportion of the "F_1 results" falling in category (b), i.e., phenocopies and mimics, is likely to be quite different in an insect and a mammal and in results from irradiation of spermatozoa and spermatogonia. Also the proportion of category (c), the inviable and sterile variants, that is comprised of individuals that do not represent point mutations at the specific loci is likely to be different in results from irradiated spermatozoa and spermatogonia. It may be concluded that when mutation rates for irradiated Drosophila spermatozoa have to be compared with mutation rates for irradiated mouse spermatogonia, the method proposed by Ives, that of using "F_1 results," may lead to serious error.

Considerable space has been devoted to criticizing the above method of comparison because Ives proposes it as the one that should be used in the future. The method that should be used, whenever possible, is to compare the species on the basis of tested mutations. When the comparison is made between data from irradiated spermatozoa in Drosophila and from irradiated spermatogonia in the mouse, another proviso must be added. This is discussed in the next paragraph.

In my original comparison (Russell, 1951) of specific-loci mutation rates in Drosophila spermatozoa and mouse spermatogonia, allowance was made for the possibility that the Drosophila spermatogonia rate might be lower than the Drosophila spermatozoa rate. On the basis of the data then available, which did not include specific-loci data, the spermatogonia rate was taken as one-half of the spermatozoa rate. Ives objects to this, stating: "I do not feel we know enough about the whole situation yet to justify that procedure." The procedure was justified by the necessity for obtaining as good estimates of human hazards as possible from the existing data. It was recognized that the "situation" had not been adequately explored and, as has already been stated, Alexander's work was started in our laboratory to help remedy the situation. It is hard to see how Ives can justify his own procedure of completely ignoring Alexander's results on this point. Alexander's work provided not only the first data in Drosophila on spermatogonia mutation rates for specific loci, but also data on spermatozoa for the same set of loci. These data show that the spermatogonia rate is, indeed, significantly lower, the point estimate being about one-fourth of the spermatozoa rate. The recent work of Auerbach (1954) indicates a similar magnitude of difference between spermatogonia and spermatozoa rates of mutation to autosomal lethals in Drosophila. Other data of both Auerbach (1954) and Muller (1954) show that certain late stages of spermatogenesis have even higher induced mutation rates than spermatozoa. Therefore, the differential between these stages and spermatogonia is even greater than that between spermatozoa and spermatogonia. Some of the Drosophila data originally regarded as spermatozoa data (and, for convenience, referred to as such in the present paper) may have been comprised partially of data from these more sensitive stages. In any case, all the indications are that the figure of one-half used for the original adjustment of the Drosophila spermatozoa rate for comparison with the mouse spermatogonia rate tended to underestimate the difference between the species rather than, as Ives implies, overestimate it.

Limitations are inherent in any method by which species can be compared. The specific-loci method was chosen for the first phase of the mouse mutation rate studies because it was felt that it would provide the most suitable type of information for species comparisons. However, my 1951 paper did not, by any means, ignore the limitations of the results obtained by this method. In discussing the limitations, Ives states: "It is evident that in both organisms some loci mutate more frequently than others and that an average rate based on only a few loci, including one which accounts for nearly half of the observed mutations, is a statistic of very limited meaning." Although Ives does not mention it, these factors, the variation among loci, the small number of loci tested, and other limitations, for example the fact that the loci were not a random sample, were all pointed out in my 1951 publication. However, it is perhaps worth inquiring further into the meaning of the statistic. Of course the particular values of the means arrived at by Ives are based on the two unjustified procedures already discussed, namely: first, the comparison of unadjusted Drosophila spermatozoa mutation rates with mouse spermatogonia rates, and, second, the use of "F_1 results." His conclusion that the two species have "similar" radiation-induced mutation rates is, therefore, based primarily on these errors rather than on any limitation of the statistic. Comparing Alexander's spermatogonia results for tested mutations with the mouse results, there is no question of a highly significant difference between the mean mutation rates of the two species so far as the loci tested are concerned. Statistical tests of high sensitivity are not available for the more important question of whether the data indicate a significant difference in over-all mutation rates between the two species, assuming the two sets of loci to be, at least, random samples of comparable kinds of loci. Nevertheless, the application of a non-parametric test, namely, Pitman's (1937), does show a difference of this kind which is significant at the 3.9% level.[3] The following semi-quantitative considerations are also instructive. Five of the seven loci tested in the mouse spermatogonia gave induced mutation rates higher than the highest of the rates obtained by Alexander for the eight loci in Drosophila spermatogonia and also higher than the highest Drosophila spermatozoa rate, even without adjusting for the four-fold greater mean rate in spermatozoa. In the quotation cited earlier in this paragraph, and in another place in his paper, Ives emphasizes the high mutation rate at the *S* locus in the mouse, apparently implying that this locus in particular gives a spuriously high value to the mean mutation rate in the mouse. However, the rate at this locus is only a little over twice that at the locus with the next highest rate. This difference is not particularly noteworthy in view of the fact that the total range in the published data is from zero mutations at two loci to 25 mutations at the *S* locus. It remains to be seen whether there are many loci with rates as high as that at the *S* locus, but the fact that, in a sample of six other loci, three have rates between one-fifth and one-half as high indicates that the *S* locus may not be entirely out of line with the other loci. As was suggested in my 1951 publication, there could be unusual features about the *S* locus, but it would be wholly unjustified, in using the present data for the estimation of human hazards, to exclude the *S* locus simply because it has the highest mutation rate of the loci tested.

In further discussing the *S* locus, Ives makes an invalid comparison of it with the *scarlet* locus in Drosophila. Thus, in comparing his results with Alexander's, he makes the statement: "Most of the difference between those two rates is accountable by the difference in *scarlet*. In Russell's mouse study there was also one locus, *spotted*, which mutated frequently. . . ." As has been pointed out, the "difference between those two rates" is attributable mainly to Ives' erroneous way of calculating rates, namely, on the basis of "F_1 results." For the seven loci in Alexander's data that can be compared with Ives' results, *scarlet* "variants" formed the largest group in her "phenocopies and mimics" category, but these were variants that had been tested and found not be to mutations at the *scarlet* locus. Alexander found no *scarlet* locus mutations in her spermatogonia study, and, on the basis of tested mutations, *scarlet* was not the most mutable locus in her spermatozoa study. *Scarlet* was also not the most mutable locus in the results of Patterson, of Mickey (1954) and of Ives. Ives' comparison of *scarlet* in Drosophila with the *S* locus in the mouse is, therefore, invalid. The purpose for which Ives makes this comparison is not clear, but if he is implying that the *S*-locus mutations in the mouse could be mostly phenocopies or mimics, this is also invalid. All spotted variants classified as presumed *S*-locus mutations that have been tested, and these now include all the ones listed as untested in the 1951 publication, have proved to be mutations at the *S* locus.

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[3] I am grateful to Dr. A. W. Kimball of the Mathematics Panel of the Oak Ridge National Laboratory for suggesting this test and making the computations.

Ives does not discuss in detail the original comparison of mouse and Drosophila mutation rates in my 1951 paper. However, in view of a statement he makes about the mouse data, and in view of what has been said in the present paper about "F_1 results," it seems desirable to reiterate the way in which the original comparison was made. It will also be of interest to consider this comparison in the light of information obtained subsequently. Ives' statement is: "Russell included in his fifty-four mutants a group of six which died before being tested for mimetic dominant mutation and a group of twelve which had not been tested at the time of publication, these eighteen cases being judged solely on the basis of the F_1 phenotype and Russell's very large previous experience with mouse mutations." This problem was discussed at length in my publication and the main reason, which Ives does not mention, for including these cases in the calculation of mutation rate was, as I then stated it: "The reliability of classification by phenotype has been confirmed by the allelism tests that have been made. In all of the first 26 presumed mutations adequately tested, the classification by phenotype has proved correct, and in the next 10 partially tested there is so far no evidence against the allelism assumed." Thus, there was strong evidence at that time that most, if not all, of the 48 viable variants classified by their phenotypes as presumed mutations at the specific loci would probably test to be such. (Since that time, all of them have indeed proved to be what they were presumed to be.)

The most extensive Drosophila data of a comparable kind at that time were those of Valencia and Muller (1949). These were published only in abstract form and it was unfortunately not possible to tell exactly whether or not the mutation rates given, which were based on mutations "from which stocks were obtained," could be compared directly with the tested mutations category in the mouse. Valencia and Muller did, however, give the further information that the mutations from which stocks were obtained represented "some 50 per cent of total." I, therefore, adopted the conservative procedure, so far as demonstrating a difference between the species was concerned, and doubled the mutation rate given by Valencia and Muller. This, after making an allowance for the difference between spermatozoa and spermatogonia results, also, as it has turned out, conservative, was compared with the total rate in the mouse, that is, the rate obtained by including the variants not yet tested at that time and the six variants that died before testing. That this was indeed a conservative procedure is now shown by the fact that, while the ratio of total variants to mutations from which stocks could be obtained was 2 in the Valencia and Muller Drosophila data, it has turned out to be only 1.1 in the mouse. Thus, the original comparison gave a mouse to Drosophila mutation-rate ratio of 12.5; but if the mouse data could have been compared with the Drosophila data of Valencia and Muller on the basis of mutations from which stocks were obtained, and if we had taken the Drosophila spermatogonia mutation rate as being only one-quarter of the spermatozoa rate, the mouse rate would have come out 47 times higher than the Drosophila rate of Valencia and Muller.

This figure is considerably higher than the best estimate of the ratio of mouse to Drosophila mutation rates that will be presented later. One possible explanation, out of many, is that there may be a difference between mutation rates for autosomal and X-chromosome loci. Before leaving the discussion of the original comparison of mouse and Drosophila mutation rates, it may be pointed out that this possibility of a difference between mutation rates for autosomal and X-chromosome loci, a possibility greatly emphasized by Ives, was recognized in three ways. In the first place, some Drosophila data on autosomal loci ware considered in the comparison. Second, the comparison was made in a conservative way and the results were stated in cautious terms. Third, we initiated the investigation made by Alexander to obtain data that would be based on autosomal loci.

Since, in Alexander's data on Drosophila spermatogonia, we now have the most closely similar results for comparison with the mouse data, it is of interest at this time to examine the magnitude of the difference in rates. The mouse rate for tested mutations may be calculated on the basis of the viable variants reported in my 1951 publication since, as mentioned above, the ten that had been only partially tested at that time and the twelve presumed mutations that had not yet been tested have since been fully tested and all of them have proved to be mutations at the specific loci. This leaves the six mouse "mutants" that died before testing. If these are excluded for purposes of comparison with Alexander's data, the mouse radiation-induced mutation rate is reduced from the published figure of 25.0×10^{-8} to 22.1×10^{-8} per roentgen, per locus. Comparing this with Alexander's figure of 1.52×10^{-8} per roentgen, per locus for the Drosophila spermatogonia mutation rate gives an estimate of 15 as the ratio of the mouse rate to the Drosophila rate.

Since the Drosophila spermatogonia mutation rate for specific loci is based on only ten mutations, it is still of interest to compare the mouse rate with the Drosophila spermatozoa rate, particularly the new and independent sets of information on specific loci in autosomes. Alexander's rate for 71 tested mutations in spermatozoa is 5.98×10^{-8} per roentgen, per locus. Mickey's (1954) rate for 32 mutations at the same eight loci is 3.35×10^{-8}. Patterson's rate (quoted by Alexander) for a total of 70 mutations at seven of the same loci and one other locus is 4.39×10^{-8}. In estimating spermatogonia rates from these spermatozoa rates, a step necessary before comparison with the mouse data, it would defeat out purpose of getting estimates independent of the limited information on specific loci rates in Drosophila spermatogonia if we were to use Alexander's estimate of the ratio of spermatozoa to spermatogonia rates. The information that comes closest to providing a satisfactory independent estimate of this ratio is that of Auerbach (1954). Table 1 in her publication gives the rate of mutation to recessive lethals on the second chromosome in broods of Drosophila coming from irradiated males mated for successive periods of three days each. Data from the last brood are presumed to be from irradiated spermatogonia. In Mickey's experiment the males were mated for six days following irradiation. His data may, therefore, be taken as being roughly equivalent to the first two broods of Auerbach's data. The ratio of the weighted mean mutation rate for these two broods compared with the rate in the last brood is 4.6. Dividing Mickey's rate by this, we come out with 0.73×10^{-8} per roentgen, per locus as an estimate of the spermatogonia rate in Drosophila. Dividing the mouse rate by this adjusted Drosophila rate gives a ratio of mouse to Drosophila rates of 30. In Alexander's experiment the males were mated for four days following irradiation. Taking this as corresponding to Auerbach's first brood and one-third of her second brood, we arrive at 3.7 as the figure with which to divide Alexander's spermatozoa rate. This gives an estimate of 1.6×10^{-8} per roentgen, per locus for the spermatogonia rate, and 14 as the ratio of the mouse to the Drosophila rate. These last figures are in excellent agreement with those obtained directly from Alexander's spermatogonia data. It may be noted that Alexander's data when compared with the mouse results give a more conservative estimate of the difference between mouse and Drosophila mutation rates than do Mickey's data. The mating period for the males is not given for Patterson's data.

Before long we hope to have adequate data on spermatozoa mutation rates in mice for direct comparison with the Drosophila spermatozoa rates. They were not obtained earlier because it was felt that it was far more important, from the human hazard point of view, to obtain spermatogonia rates. In this connection, Ives makes another error. He states: "In mice the mature sperm are so affected by the radiation that the zygotes resulting from them are abortive. Therefore one does not know the actual mutation rate at the visible loci of mice in mature sperm....." This is incorrect. At 600 r, dominant lethality in mice is not too great to permit determination of mutation rate in sperm. This has been known for over twenty years (see review by Russell, 1954).

Ives nowhere discusses the application of the mouse-Drosophila comparison to the estimation of human hazards. In my 1951 paper the species comparison was made primarily with this estimation in mind and was, in fact, discussed under the section heading "Human Hazards." I concluded this section with the following statement: "From a cautious scientific point of view, it would be unwise to generalize....but, from the point of view of those concerned with the immediate problems of protection in man, it would be risky to ignore the indication that estimates of human hazards based on Drosophila mutation rates may be too low." I still hold this point of view. Comparisons between species as different as Drosophila and mice are difficult. There are many possible complicating factors additional to those already discussed. To mention only one, the relation between mutation rate and dose, for recovered mutants from spermatogonia in the mouse, is proving to be non-linear. Because of the possible complicating factors, it would still be unwise to generalize about the relative rates of mutation in Drosophila and mice, although one can perhaps be less conservative than I was in my 1951 publication. However, because of the human hazards, there is a pressing need to draw the best conclusion one possibly can. It is, therefore, important that, regardless of the difficulties, the comparison between Drosophila and mice be made as meaningfully and as accurately as possible at the present time. It is on this basis that Ives' arguments and conclusions have been questioned, and not because of any belief that a final answer has been reached as to the relative mutation rates of the two species.

A casual reader of Ives' paper, if he accepts Ives' conclusion that "the radiation-induced mutation rate per r per locus appears to be similar in flies and mice," might think that this invalidates the conclusion of my 1951 paper that "estimates of human hazards based on Drosophila mutation rates may be too low." Such is not the case. Ives does not have any serious objections to the figure of 25×10^{-8} per roentgen, per locus, for the mouse mutation rate; he is simply "calculating" the Drosophila rate in a way that raises it closer to the mouse rate. But the Drosophila figure that was being used most commonly to estimate human hazards at the time when my 1951 paper suggested more caution, was not the high figure of 14×10^{-8} or 23×10^{-8} per roentgen, per locus, arrived at by Ives, but 3×10^{-8}. Furthermore, since this was based on Drosophila spermatozoa, it was also assumed that there might be a safety factor of two or so in applying this to human spermatogonia. Thus, regardless of whether Ives' conclusions are correct or not, and the burden of this paper has been to demonstrate that they are not, he has not shown any reason to relax from the extra caution that the mouse results originally indicated should be taken.

CONCLUSIONS

In an earlier paper (Russell, 1951) it was concluded that comparison of our data on radiation-induced mutation rates in mice with similar data from Drosophila, particularly those of Valencia and Muller (1949), indicated a considerably higher mean induced mutation rate in the mouse. New information on Drosophila supports this conclusion. The Drosophila mutation rates now most suitable for comparison with the mouse are those obtained primarily for this purpose by Alexander (1954). These rates, like the mouse rates, are for mutations at specific loci on autosomes and they were obtained by essentially the same genetic technique and method of scoring the mutations as had been used on the mice. Furthermore, the X-ray machine and method of dosimetry were the same as those used in the mouse experiments. Perhaps the most important new feature of this Drosophila information is that it was obtained from irradiation of the same cell stage as that investigated in the mouse, namely, the spermatogonial stage. Comparing the mouse' radiation-induced mutation rate based on genetically tested mutations with these comparable data in Drosophila gives an estimate of 15 as the ratio of the mouse rate to the Drosophila rate.

The general magnitude of this ratio is also supported by several recent estimates of Drosophila mutation rates for autosomal loci in postspermatogonial cell stages, provided due regard is paid to the differences between cell stages. Ives' (1954) conclusion that the radiation-induced mutation rate "appears to be similar in flies and mice" is disputed on several grounds.

Comparisons between species as different as Drosophila and the mouse are difficult. It is not maintained that a final answer has been reached on the relative radiation-induced mutation rates of the two species. However, the new Drosophila data would seem to reinforce the conclusion reached in my 1951 paper that "from the point of view of those concerned with the immediate problems of protection in man, it would be risky to ignore the indication that estimates of human hazards based on Drosophila mutation rates may be too low."

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INCREASE OF LEARNING CAPABILITY WITH INCREASE OF BRAIN-SIZE

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Many lines of descent, especially in mammals, show a successive increase of body size (Cope's rule) and hence also an increase of *absolute* brain size. But normally the *relative* brain size is less in larger species. This latter rule, already discovered by A. von Haller in 1762, may be explained by the fact that most innervated parts of the body, especially the outer and inner surfaces, grow by the square, but the central nervous systems by the cube. Therefore the brain of larger animals may be relatively smaller without diminishing its central nervous functions. Nature "tries," so to say, to keep the nervous functions practically alike. But apparently with increase of body size the persistence of a totally balanced structural brain type becomes difficult, because the relative growth ratios of practically all brain regions are different and will hence shift the proportions of the brain.

Moreover in long lines of descent we often find Haller's rule superimposed upon another rule indicating that there exists also a special, progressive evolution of the brain, by which the relative brain size is sometimes increased. This rule of progression especially applies to the highest nervous centers, in insects chiefly to the Corpora pedunculata, in higher vertebrates particularly to the forebrain (cf. for example, the skull casts of the horse line: Edinger, 1948). The progression is not only evident from the quantity of neurons and of their arrangement, but also from their improved properties. In most cases the neurons became more densely arranged in the course of evolution and form more special centers, and the cells partly develop a richer ramification. The sensory neurons generally remain very small, but the number of them is considerably enlarged. The detailed perception of complex stimuli brought about by physiological processes of the sense organs can thus be separately represented also in the central nervous systems. Thus it became easier to react specifically to small parts of a complex stimulus pattern. On the other hand a more detailed formation of engrams became possible, and the perception and retaining of a Gestalt became easier. Probably these advantages proved decisive in the parallel development of very numerous, particularly small sensory neurons in the three most progressive types of brain, in cephalopods, in insects and in vertebrates. On the contrary motor neurons often became especially large, because of the motor reactions to complexes of different stimuli required to comprise many excitations in the sense of a resultant and to conduct the motor excitation quickly to the effectors.

But now as absolute body size and therefore a certain brain size connected with it is an important selective (mostly not a genetical) unit, we will take no account of the share of Cope's and Haller's rule and the rule of progression in the single cases, and we will only examine the importance of different absolute brain size in phylogeny. When we compare large and small brains of related animals of different body size as a model of a real line of descent following Cope's rule we can note strong histological differences at first, because the single regions show different growth ratios, being positively or negatively allometrical. During the last years we have been working on these problems in more detail in our Zoological Institut in Münster. As I already have summarized a large part of the results (Rensch, 1954 a, b), I may restrict myself to the most important and to the more recent results.

INVERTEBRATES

In different orders of insects H. Goossen (1949) showed that most large species have more complicated Corpora pedunculata than related smaller species, that is the Corpora pedunculata show more or deeper foldings in the frontal side of their neuropil (which in some respect are parallel to the foldings of the mammalian cortex). The number of globuli-cells is always much larger in larger species, for example, in a maximum section (10μ thick) of the Corpora pedunculata this number was 615 in the large water-beetle *Dytiscus marginalis* (length 27 mm), but only 188 in the small water-beetle *Ilybius fenestralis* (length 12 mm). Probably this difference is the result of a chiefly positive allometrical growth of the Corpora pedunculata, being effective in holometabolic insects mostly at the end of the larval development (Jonescu, 1909). In hemimetabolic insects the situation is different. In our Laboratory R. Neder (unpublished) compared the postnatal development of the brain of three species of Blattidae. He showed that in *Periplaneta americana* a period of positive allometry of the Corpora pedunculata is found in the first and second larval stages, but that this growth-gradient later turns into a weaker negative allometry. Corresponding to this fact the giant cockroach Macropanesthia from Australia shows a decrease of the relative size of the Corpora pedunculata compared with smaller species (Day, 1950).

Now, the relatively larger size of this progressive brain region, serving only for connecting nervous conductions, also affects the behavior. The larger European Vespidae and Apidae (Vespa, Polistes, Apis, Bombus, etc.)

generally have more complicated social instincts. All small species of both families, in which the Corpora pedunculata are simpler, show no social life (Symmorphus, Ancristocerus, Lionotus; Andrena, Halictus, etc.). [The brain-structure of some rather small social Apidae and Vespidae of the tropics ought to be examined.] Among ants the small Ponerinae have simpler social instincts than the larger Camponotinae, and the smallest ant-species *Monomorium salomonis* (length 1.8–2.5 mm) shows only poor social instincts. Among the Scarabaeidae the large species like Scarabaeus, Copris, Geotrupes show complicated instincts in the care of their eggs (when constructing breeding-pills from dung, subterranean canals, breeding chambers), whereas the many small species of the related genus Aphodius have not such instincts, simply depositing their eggs in dung. The same holds good for the Silphidae, in which the large species of Necrophorus show very complicated breeding instincts (by digging in the carcass, feeding of the begging young larvae), whereas the small species of the related genus Catops (only 3mm long) show nothing like this.

Whether the learning capability of insects depends upon the absolute brain size or not, cannot yet be decided. Some years ago I performed some training experiments and at first I had some positive results, (Rensch, 1949), showing at least longer retention in the larger species. But the sources of errors still were too large and the experiments must be repeated.

VERTEBRATES

But in vertebrates the relations between absolute brain size, histological brain structure and learning capability are rather striking. These relations are of interest, because they contribute to a selective explanation of Cope's rule of general phyletic increase of body size. An analysis of these relations required: 1) histological studies on the growth ratio of the single regions and areas of the brain during ontogeny; 2) corresponding histological inquiries upon the differences of adult brains in related species of different body size (as a model of the real lines of descent); 3) corresponding inquiries upon sense organs, and 4) upon hormone glands; 5) comparative inquiries upon instincts, capabilities of learning, retaining and generalizing in related animals of different body size. Up to the present we could only tackle a part of such a large program, but we already have some more general results.

First Harde (1949) showed that in the forebrain of white mice the single regions and areas show specific growth ratios. Thus the cytoarchitectonic proportions differ at each ontogenetic stage, and sometimes also a reversal of the direction of allometry may occur. For example, the holocortex 5-stratificatus grows mostly with positive allometry (in relation to the whole cortex) until the 13th postnatal day, that is, the day of opening the eyes, but later on with negative allometry.

Now, a comparison of adult rodents of different body size shows that some of these growth gradients have remained similar in the different species, whereas others have been markedly altered in phylogeny (Schulz, 1951). But apparently the relatively largest and most progressive brain region, the holocortex 7-stratificatus more or less maintains its direction of allometry, being positive until maturity, in ontogeny as well as in phylogeny. In the series white mouse-white rat-rabbit this region increases from 37.2% to 39.8% and 42.0% of the whole cortex (differences statistically valid). The semicortex on the contrary, growing isometrically or with negative allometry, decreases in the above series: mouse 18.7%—rat 13.9%—rabbit 9.0%.

Quite recently similar inquiries on races of domestic fowl of different body size were performed. Schlabritzky (1953) showed that in five races of different size the correlation of brain weight to body weight remains constant in ontogeny, that is, young individuals of dwarfs have the same relative brain weight as young chickens of giants being of equal body size. In spite of this fact the histologically different brain regions do not grow isometrically with the whole brain, but show different growth gradients. Especially the Neostriatum, which is so typical for the bird brain, grows with positive allometry (figure 1), whereas the cortical regions grow with negative allometry (figure 2). (Krumschmidt, in press). Hence also in closely related birds, for example, races of domestic fowl of different body size, marked differences exist in the proportions of the forebrain and therefore differences in behavior can be expected. The same holds good for lizards (Rose, unpublished).

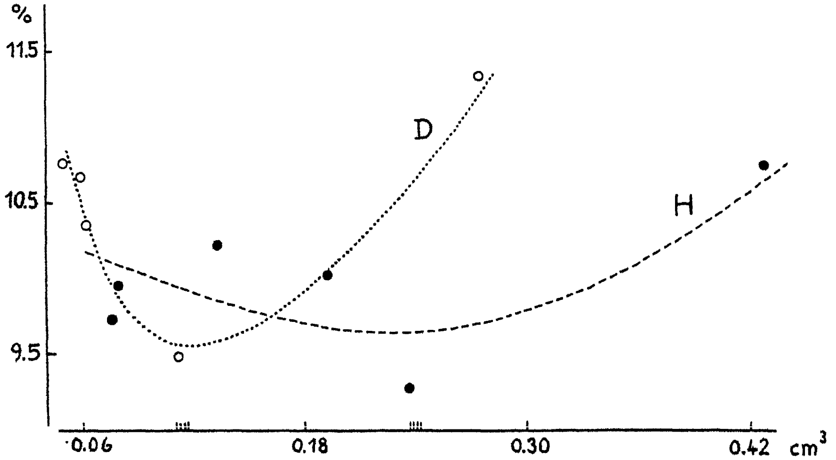

FIGURE 1. Correlation between the relative volume of the neostriatum (ordinate) and the absolute volume of the striatum (abscissa) in races of domestic fowl. H = New Hampshire, D = dwarfs. (After Krumschmidt).

Also the postnatal growth of the forebrain of newts is not isometrical in all its parts, but the Area dorsalis and the A. medialis grow more or less with positive allometry. The Striatum on the contrary grows with negative allometry, while the Epistriatum grows isometrically (Homeyer, 1951). Corresponding to this fact, related species of newts and salamanders show different proportions of their brain regions: in large species the Area dorsalis is relatively larger, and in the mid brain the cell layers of the Tectum opticum are more numerous (Nolte, 1953).

As an example taken from fishes Wellensiek (1953) analyzed the brains of Cyprinodontidae of different body size. Here the smallest species have a relatively large mid brain and a relatively very large Tectum opticum, whereas the forebrain is relatively small.

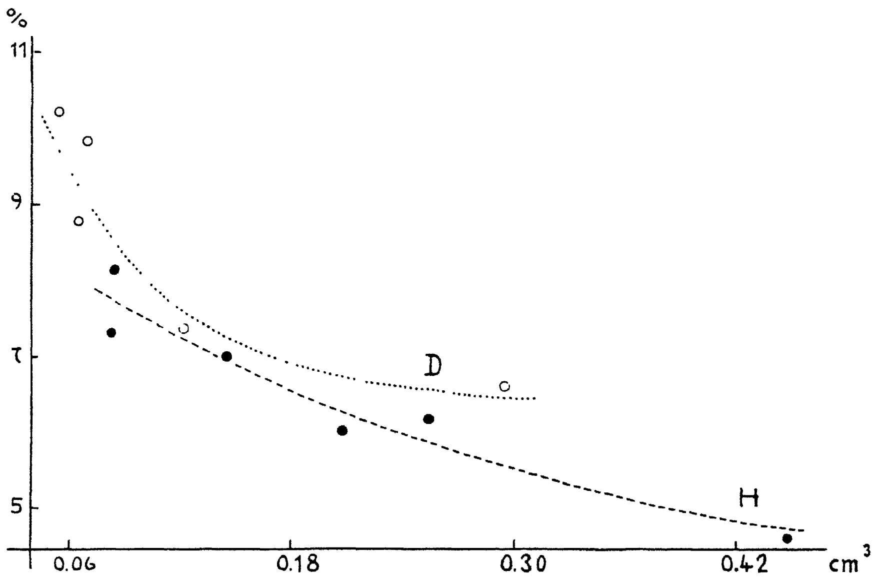

FIGURE 2. Correlation between the relative volume of cortical areas (ordinate) and absolute volume of the hemisphere (abscissa) in races of domestic fowl. H = New Hampshire, D = dwarfs. (After Krumschmidt).

Now, all these histological differences in larger and smaller species of the same relationship are further increased by cytological differences. Generally, in larger species the neurons of the brain are absolutely larger and the ganglionic layers are less wide than in smaller species. The neurons also show more dendritic ramifications, which are particularly conspicuous in pyramidal cells, but also in nerve cells of the body (Levi, 1925; Bok, 1936; Bok and van Erp Taalman Kip, 1939; Rensch, 1947; 1949; 1953; 1954; Nolte, 1953; Spina Franca Netto, 1951; Tower, 1954).

Furthermore it is important for the evolution of behavior that the increase in body size by selection (Cope's rule) often improves the function of the sense organs. Here it may be sufficient to refer to the histological structure of eyes. Normally large species have more sense cells than smaller species (among insects more ommatidia), and the percentage of rods and

TABLE 1

TOTAL NUMBERS OF NEURONS AND SENSE-CELLS IN THE RETINA OF FOUR SPECIES OF EUROPEAN NEWTS AND SALAMANDERS OF DIFFERENT BODY SIZE. (AFTER A. MÖLLER).

Species	Diameter of eye	Total number of ganglion cells	Total number of rods and cones
Triturus vulgaris	2,2mm	193 600	171 300
Triturus cristatus	2,7mm	207 500	224 300
Salamandra atra	3.3mm	316 500	386 300
Salamandra maculosa	5,5mm	308 300	533 000

cones and the numerical relation of sense cells, bipolars and cells of the ganglionic layer is shifted parallel with body size (table 1). This holds good for fishes, amphibians, birds and mammals (Bucciante and de Lorenzi, 1930; Burckhardt, 1931; Rensch, 1947; 1948; 1954; Möller, 1950; Müller, 1952, Wellensiek, 1953). Larger species thus show a better dissolving capability of the retinal image and perceive smaller parts of a complex stimulus pattern. Moreover the flatter lens of larger mammals allows a better accomodation (Rensch, 1947, 1948, 1954).

Finally, we have to take into consideration that a selection which alters body size, also alters the relative size of many hormone glands influencing the behavior, (Jackson, 1913; Riddle, 1927, Oboussier, 1948; Padour, 1950; Schlabritzky, 1953).

EFFECTS ON VERTEBRATE BEHAVIOR

Now, all the above differences between larger and smaller related animals, differences of the relative size of certain brain parts, of single cytoarchitectonic regions, of the size of neurons and of their ramification, of sense organs and of hormone glands can affect the behavior. To prove this, we compared large and small related species or races of fishes, birds and mammals and got more or less similar results in all groups.

Larger species are generally slower, more "thoughtful," more steady in behavior, because of their less intense metabolism (Rensch, 1947, 1954). This holds good for Cyprinodontidae, for races of domestic fowl, for large and small parrots, for rodents, etc.

At least in some cases the instincts of larger species are more complicated than those of related smaller species. Among grouse the courtship is more complicated in Tetrao and Lyrurus than in the smaller Tetrastes; in the large bustard *Otis tarda* more complicated than in the small *O. tetrax*; in the large grebe *Podiceps cristatus* more complicated than in the small *P. ruficollis*. Young rats show playing instincts, young mice do not play (Eibl-Eibesfeld). But with respect to the enormous complication of brain structure in all vertebrates, also in small ones, it is doubtful, whether a corresponding rule of more general applicability exists.

But the learning capacity is generally larger in larger species. In Cyprinodontidae of different body size (Xiphophorus and Lebistes) the larger species did not learn more visual tasks, but the experiments have to be repeated with more individuals (Rensch, 1954 a,b,c). Altevogt's investigations in races of domestic fowl of different body size and thus different absolute brain size (1951) were rather striking. Large races succeeded in mastering six visual tasks (discrimination pairs like red against green, black circle against black triangle, square against cross, etc.). A dwarf race only mastered four such tasks (one individual five tasks for a short time). Apparently there are similar differences between related species of different body size of other families of birds, as the (averbal) counting experiments by Koehler and his school proved. The best budgerigar (Melopsittacus) of Marold (1939), for instance, learned to eat 2, 3 or 4 grains according to three differently colored patterns arranged in front of the food boxes. But a large Amazon parrot of Brauns (1952) even learned to find the right pattern out of five food boxes corresponding to either three, four, five, six or seven spots on a cardboard shown as a "signal" in front of the food boxes. And in these experiments the spots on the "signal" cardboard needed not have the same size and arrangement as those on the cardboards covering the food boxes. A similar task was mastered by a raven of Koehler (1943), whereas the smaller jackdaws of Schiemann (1939) only learned to eat two or four mealworms, when a similar "signal" cardboard indicated two or four spots.

A corresponding comparison of rats and mice did not reveal such differences in the quantity of learned tasks during the first series of experiments (von Boxberger, 1953). Both species learned to discriminate six pairs of patterns arranged successively in a running way. They had to pass a flap door with the positive pattern six times and had to avoid a locked door with the negative pattern. The succession of patterns and the positive side were always changed. When a seventh task was added, neither species succeeded. But as the rats ran more "blindly" and the mice were more cautious and as they were more attracted by the food, I had Miss W. Reetz repeat these learning experiments with similar patterns, but applying Lashley's jumping method, because here the psychological conditions were more alike for both species. The size of the patterns and the jumping distance were chosen proportionately to the body size of rats and mice. It was interesting that these experiments had other results (still unpublished). Now the rats learned nearly all tasks more quickly than the mice, and they also learned more tasks. In the serial rotation test with five tasks both species gave nearly equal results, but the test with seven tasks was mastered by all five rats, but only by one of the mice. Three rats even learnt eight tasks (data statistically valid), whereas the last mentioned mouse had forgotten one of the tasks (table 2).

As the learning capacity seemed to be correlated with the absolute brain size, it was of interest to examine the terrestrial mammal having the larg-

TABLE 2

MULTIPLE TESTS OF RATS AND MICE. IRREGULAR SERIAL ROTATION OF 6–8 PAIRS OF PATTERNS. EACH PAIR TESTED 50 TIMES IN EACH SERIES. PERCENTAGE OF RIGHT CHOICES. (AFTER W. REETZ).

Patterns	6 tasks		7 tasks		8 tasks	
	5 rats	5 mice	5 rats	1 mouse (of 5)	3 rats (of 5)	1 mouse (of 5)
square-cross	85	86	91	84	90	76
white-black point	94	96	98	84	96	98
wave-stripe	82	80	87	72	91	(38)
dark-white ring	75	79	85	84	82	94
triangle-2 stripes	85	75	82	90	92	80
coarse-fine stripes	99	96	99	100	99	100
cheque-oblique spot	...	..	82	84	92	78
angle-points	.	. .	...	. .	97	94

est brain (about 6000 g), the elephant. And indeed, the capacity was surprisingly great in this animal (Rensch and Altevogt, 1953, 1955). We trained a young female Indian elephant (5–6 years old) to discriminate between a pair of patterns, positive choice rewarded. The first task was a black cross against a black circle. As soon as the task was learned we trained the animal to discriminate a new pair of patterns. After several pairs of patterns had been learned, we tested the animal, whether it mastered all tasks in serial rotation, when patterns and positive side were

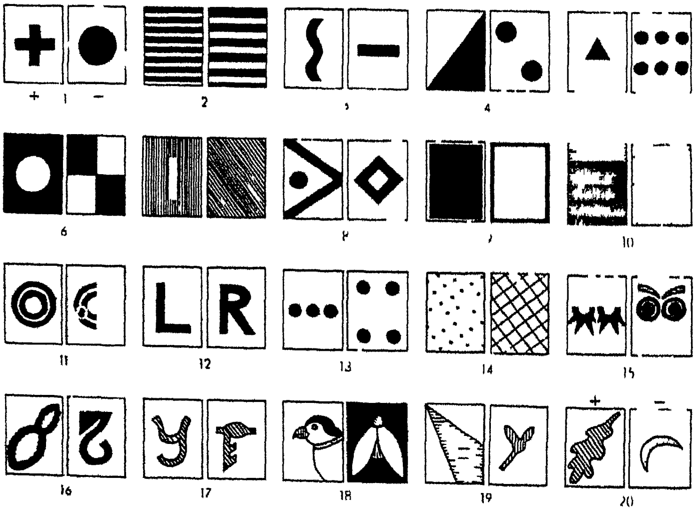

FIGURE 3. 20 pairs of patterns, which a young Indian elephant mastered in serial rotation. Each positive pattern to the left. (After Rensch and Altevogt).

TABLE 3

MULTIPLE TEST OF YOUNG ZOO-ELEPHANT. 20 LEARNED PAIRS OF PATTERNS IN IRREGULAR SERIAL ROTATION. 30 TRIALS OF EACH PAIR OF PATTERN. (AFTER B. RENSCH AND R. ALTEVOGT).

	Pairs of patterns			Percentage of right choices
1.	cross	against	circle	93% ±4.7
2.	fine	"	coarse stripes	100%
3.	snake line	"	stripe	87% ±6.1
4.	oblique triangle	"	2 points	100%
5.	triangle	"	6 points	97% ±3.1
6.	white point	"	cheque	100%
7.	red	"	green	83% ±6.9
8.	angle	"	rhombus	80% ±7.3
9.	black	"	white	97% ±3.1
10.	blue	"	yellow	100%
11.	double ring	"	2 half rings	100%
12.	L	"	R	80% ±7.3
13.	3 points	"	4 points	100%
14.	many points	"	grid	93% ±4.7
15.	closed	"	opened eyes	97% ±3.1
16.	8	"	z	100%
17.	y (green)	"	note (green)	93% ±4.7
18.	parrot	"	snowdrop	100%
19.	blue wedge	"	red hare	87% ±6.1
20.	oak leaf	"	crescent	97% ±3.1

changed irregularly. Finally, the elephant mastered 20 pairs of patterns (figure 3). In one large test she stood 600 trials resulting in 80–100% correct choices for each pair of patterns (data statistically valid, table 3). By simultaneous presentation of one positive and three negative patterns we could prove that the elephant knew all the 40 patterns singly as positive or negative ones (figure 4). Another co-worker has just begun comparative experiments with other ungulates.

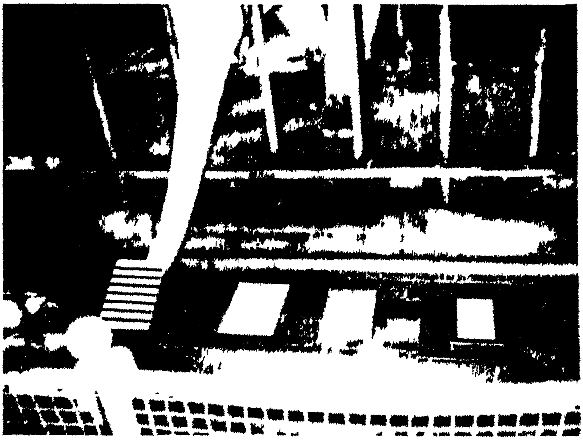

FIGURE 4. Simultaneous presentation of one positive (stripes) and 3 negative patterns (crescent, green, white with black margin) to an Indian elephant. (After Rensch and Altevogt).

Corresponding with this astonishing learning capacity, also the acoustic learning ability is very highly developed in elephants, as we could show in India (Rensch and Altevogt, 1955 b). Older animals between 30 and 60 years of age react to 21-23 different verbal commands (given in Urdu language) by purely auditory perception. Their corresponding actions often were very plastic and adapted to the special situation. At present the zoo elephant mentioned above has learned to discriminate between 12 different tones produced by a tone generator three of them differing only by one step), six of them being rewarded. (Reinert, unpublished).

But large animals also retain for a longer period than related smaller species. This could already be shown in Cyprinodontidae. Two series of *Xiphophorus helleri* (two and five specimens) retained a learned task (.... against :) for 54 and 30 days (on the average); two series of the smaller *Lebistes reticulatus* (four in each series) not even for three days (Rensch, 1954 b) (table 4). After 20 days without training a giant race of domestic fowl mastered six pairs of pattern, learned before, whereas a dwarf race only mastered three-five tasks. Five younger white rats retained a visual task three weeks longer (on the average) than five white mice of a physiologically similar age. The best rat trained by Reetz retained eight pairs of patterns for 33 days, four pairs of patterns for 154 days, one pair of patterns for 459 days, whereas the best of the correspondingly trained mice only mastered four pairs of patterns after 103 days and one pair of patterns after 195 days (controlled at intervals of four weeks; always 10 trials without reward). After a period of one year without training the zoo-elephant recognized 12 tasks of 13 tasks learnt before, but also in the 13th task she made 67% of right choices, this datum being nearly statistically valid (520 choices within three hours, each pair of patterns presented 40 times in an irregular sequence, table 5).

TABLE 4

RETAINING TEST IN TWO SERIES (A,C) OF CYPRINODONTIDAE OF DIFFERENT BODY SIZE. TRAINED TASK: FOUR POINTS HORIZONTALLY (POSITIVE) AGAINST TWO POINTS VERTICALLY (NEGATIVE). AVERAGES OF PERCENTAGE OF RIGHT CHOICES. EACH TIME 10 TRIALS WITHOUT REWARD.

Days after last training experiment	3	17	27	44	54	64
A. 2 Xiphophorus	75	90	85	70	80	45
4 Lebistes	67	40	35	56	27	20
Days after last training experiment	0	19	30	41		
C. 5 Xiphophorus	94	84	65	53		
4 Lebistes	92	58	60	27		

TABLE 5

RESULTS OF A RETAINING TEST OF A YOUNG ELEPHANT FOR 13 LEARNED PAIRS OF PATTERNS AFTER ONE YEAR WITHOUT TRAINING. PATTERN HERE IN SUCCESSION OF LEARNING. EACH PAIR OF PATTERNS TESTED 40 TIMES IN IRREGULAR SERIAL ROTATION. (AFTER B. RENSCH AND R. ALTEVOGT).

Pair of pattern				Percentage of right choices
1.	cross	against	circle	90% ±4.8
2.	fine	"	coarse stripes	90% ±4.8
3.	snake line	"	stripe	73% ±7.0
4.	oblique triangle	"	2 points	97% ±2.7
5.	triangle	"	6 points	100%
6.	white point	"	cheque	95% ±3.4
7.	red	"	green	95% ±3.4
8.	angle	"	rhombus	67% ±7.5
9.	black	"	white	80% ±6.3
10.	blue	"	yellow	97% ±2.7
11.	double ring	"	2 half rings	77% ±6.7
12.	L	"	R	73% ±7.0
13.	3 points	"	4 points	83% ±5.9

In transposition experiments, that is, by alteration of the learned patterns, the rats of Miss Reetz were markedly better in five tasks than the mice, the latter were only better in one task. The elephant was also able to recognize far-going alterations of the learned patterns. In the Cyprinodontidae corresponding experiments did not show clear differences between the larger and the smaller species.

SUMMARY & CONCLUSIONS

Summarizing all the results we may state that in vertebrates larger species, that is species with absolutely larger brains, are able to learn more (and apparently also more complicated) visual tasks and to retain them for a longer period than comparable related species of smaller size. The capability of abstraction (transposition) has not yet sufficiently been examined. The differences obtained correspond in some respect with the statements of Lashley (1931) and Layman (1936), that the capacity for learning is proportionate to the mass of the forebrain, when this is reduced by operation.

With regard to the intact animals of our experiments I would presume that an increase of the learning capacity in larger species is due to the absolutely larger neurons and the correlated increase in number of dendrites effecting a more complex switch mechanism. The capacity of larger animals to retain for a longer period is perhaps also due to the larger cell size allowing a stronger histological connection as a basis of associations. Of course many experiments will be needed to confirm the mentioned results and hypotheses.

APPLICATION TO EVOLUTION

As regards *evolution* these statements are especially important, because they show that all purely quantitative increase of the numbers and of the size of neurons can be a selective advantage, for it is doubtlessly advantageous to learn more and more complicated actions and to retain for a longer time. Thus the rule of progression of the brain becomes intelligible and one of the factors (besides others), explaining Cope's rule of successive phyletic increase of body size becomes clear.

Apparently the development of behavior follows the morphological alterations of the brain. In our case this would mean: at first probably new brain regions were developed phylogenetically by positive allometry of progressive parts, and later on these parts became successively filled with functions of behavior. Such a working hypothesis can be supported by several facts, which I will indicate only shortly.

1) In the lowest vertebrates the cerebellum is developed as an additional centre. In sharks it is already rather large, but extirpation is not very effective. In birds the Lobus medius is more developed, but does not seem to have any specific functions yet. 2) In amphibians the forebrain is already relatively large, but is still fairly unimportant, as proved by extirpation experiments. Moreover, the studies by Nolte (1953) suggest that in amphibians especially the positive allometry of the Area dorsalis, a region where no important functions seem to be localized, would be the basis of an improved development of a future cortex. 3) After centres for all senses had developed in the vertebrate brain, additional regions arose and now could be used as centres of association. The same holds good for the Corpora pedunculata of the polychaetes and arthropods as well as for the frontal and temporal lobes of mammals. This is especially so with Broca's region of the human brain, which could be filled additionally with motor functions of speech, thus contributing to the rapid development of human culture. And apparently, the brains of higher vertebrates are capable of many more functions than are usually required in their normal life. This becomes evident from the astonishing performances of trained animals, for example, the averbal counting ability of birds. Therefore all higher vertebrates perform many "unneccessary" actions during their life (sometimes similar to playing). All this seems to indicate that the enlargement of the brain normally happens by the formation of new regions, which become filled with functions only successively.

EVOLUTION OF BEHAVIOR

When we now try to view these problems from an aspect of the evolution of behavior as a whole, similar conclusions are possible. We may state that the selective alterations often happen in the sense of an "addition to the final stages." In different lines of descent, especially in vertebrates, the increasing complication often is brought about by additional complexes of neurons becoming centres of a higher order. At first the mid-brain developed as a main centre of association. Afterwards this function was shifted to the subcortical regions of the forebrain, then to the cortex. Here at first the sensory and motor regions served this function and finally the larger centres of association became active. Also in annelids and arthropods a corresponding improved development resulted in the formation of larger association centres in the Corpora pedunculata. Corresponding with this course of development at first reflexes and deflexes (plastic reflexes) developed, then rigid or more plastic instincts arose, after which actions based on learning processes (engrams), on abstractions or on insight (comprehending causal connections) could be developed. The driving force of this improvement was the advantage of an increasing adaptation to the complicated stimulus situations of the habitats, that is, to the extrasubjective realities (cf. Rensch, 1954a).

It is remarkable that very different histological systems, the midbrain of fishes, the subcortical regions of birds, and the cortex of mammals, enabled the animals to attain the same capacities of learning, retaining and abstracting. [Learning in the sense of conditioned reflexes, can already be performed by the spinal cord of amphibians: Franzisket, 1951, Rensch and Franzisket, 1954, Rensch, 1952].

If we now ask, *why* this development was more or less an addition to the final stages and not an "archallaxis" (Sewertzoff, 1931), that is, an alteration already beginning with the first ontogenetic stages, the answer will be similar to that concerning the development of other organs. After a harmoniously working structural type had developed in phylogeny, it was easier that variants survived, in which a new development was added to the final stages, because all earlier alterations would more disturb or destroy the established harmony.

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GENERATION TIME AND THE BIOLOGICAL NATURE OF VIRUSES

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INTRODUCTION

Whether or not viruses are to be regarded as organisms or chemicals is a matter of disagreement or of point of view (Stanley, 1938; Burnet, 1945). If it could be shown that the rate of reproduction of conventional organisms shows a quantitative relation to their size, and if it could be shown that our present knowledge of virus size and rate of reproduction fits into this quantitative relation for conventional organisms, this would constitute evidence that viruses are organisms. Such is attempted here.

No previous study of this type has been found. Evans and Smith (1952) give times for doubling of populations of two rodents and three insects with data which support the absolute data and the trend presented in table 1 and figure 1 of the present study, but these authors do not point out any relation between body size and generation time. Elton (1927) states the well known fact that small animals increase faster than large ones, but does not present quantitative evidence.

METHODS

Size is given as the fresh weight of the adult organism. With small organisms this is calculated from microscopic measurements and known or assumed densities. With viruses it is assumed that the bodies seen in the electron microscope are the adult forms. Generation time is given as the time for a population to double under what are believed to be near optimum conditions for reproduction. Since most organisms and some viruses reproduce in cycles with multiple progeny, the generation times do not correspond to the time for a normal generation or cycle. Generation time is calculated as:

$$\frac{\text{time X logarithm of 2}}{\text{logarithm of increase in population}}$$

from known or estimated rates of increase. Time is the interval in minutes during which increase in population was studied. Increase in population is the proportional increase in numbers. For example, Chapman (1928) found that a colony of flour beetles (*Tribolium confusum*) increased from 2 to 4500 individuals in 154 days. Time would then be 154 × 24 × 60 minutes,

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[1] The assistance of O. P. Pearson in securing the data for mammals is gratefully acknowledged.

TABLE 1

SIZE AND RATE OF REPRODUCTION OF VIRUSES AND CONVENTIONAL ORGANISMS.

Virus or organism	Body Weight	Authority for body weight	Generation time	Authority for generation time
	grams		*minutes*	
Foot and mouth disease virus	5×10^{-19}	Rhodes et al., 1953	5×10^{1}	Henderson, 1953
Tobacco necrosis virus	5×10^{-18}	Stanley, 1947	1×10^{2}	Yarwood, 1952
Southern bean mosaic virus	1×10^{-17}	Stanley, 1947	2×10^{2}	Yarwood, 1955
Tobacco mosaic virus	7×10^{-17}	Stanley, 1947	6×10^{1}	Yarwood, 1952
T_2 coli phage	2×10^{-16}	Rhodes et al. 1953	5×10^{0}	Adams 1950
T_5 coli phage	8×10^{-16}	Weigle et al. 1951	1×10^{1}	Weigle et al. 1951
Influenza virus	1×10^{-15}	Stanley, 1947	6×10^{1}	Horsfall, 1953
Escherichia coli	3×10^{-13}	Breed et al. 1948	2×10^{1}	Mason, 1935
Xanthomonas phaseoli	1×10^{-12}	Breed et al. 1948	3×10^{2}	Allington et al. 1949
Chlorella pyrenoidosa	6×10^{-11}	Burlew, 1953	2×10^{2}	Burlew, 1953
Hansenula anomala	1×10^{-10}	Lodder et al. 1952	1×10^{2}	Grieg et al. 1941
Saccharomyces cerevisiae	2×10^{-10}	Lodder et al. 1952	1×10^{2}	Pennington et al. 1951
Colpidium colpoda	5×10^{-8}	Pratt, 1916	6×10^{2}	Cutler et al. 1923
Enchelys farcimen	6×10^{-8}	Robertson, 1921	2×10^{2}	Robertson 1921
Paramecium caudatum	5×10^{-7}	Kalmus, 1931	7×10^{2}	Gause, 1934
Monilinia fructicola	8×10^{-7}	Yarwood, 1955	1×10^{3}	Yarwood, 1955
Erysiphe polygoni	1×10^{-6}	Yarwood, 1955	5×10^{2}	Yarwood, 1955
Hydratina senta	1×10^{-5}	Hudson et al., 1889	5×10^{2}	Edmondson, 1946
Brevicoryne brassicae	1×10^{-3}	Herrick, 1926	3×10^{3}	Herrick, 1926
Tribolium confusum	1×10^{-3}	Yarwood, 1955	2×10^{4}	Chapman, 1929
Musca domestica	2×10^{-2}	Yarwood, 1955	3×10^{3}	Howard, 1924
Physa gyrina	1×10^{0}	DeWitt, 1954a	2×10^{4}	DeWitt, 1954b
Passer domesticus	2×10^{1}	Pearson, 1955	1×10^{5}	Barrows, 1889
Ostrea virginica	3×10^{1}	Pratt, 1916	2×10^{4}	Galstoff, 1930
Rattus norvegicus	2×10^{2}	Pearson, 1955	8×10^{4}	Fischer, 1972
Gallus domesticus	2×10^{3}	Robinson, 1912	8×10^{4}	Robinson, 1912
Oryctolagus cuniculus	2×10^{3}	Asdell, 1946	1×10^{5}	Fenner, 1954
Phasianus colchieus	2×10^{3}	Yarwood, 1955	2×10^{5}	Yarwood, 1955
Solanum tuberosum	3×10^{3}	Yarwood, 1955	9×10^{4}	Yarwood, 1955
Helianthus annuus	6×10^{3}	Yarwood, 1955	4×10^{4}	Yarwood, 1955
Homo sapiens	5×10^{4}	Yarwood, 1955	5×10^{6}	Malthus, 1803
Sus scrofa domestica	1×10^{5}	Asdell, 1946	2×10^{5}	Asdell, 1946
Bos taurus	4×10^{5}	Asdell, 1946	1×10^{6}	Asdell, 1946
Sibbaldus musculans	8×10^{7}	Jordan, 1929	1×10^{6}	Krogh, 1934
Pseudotsuga taxifolia	2×10^{8}	Frothingham, 1909	2×10^{6}	Baker, 1950
Sequoia gigantea	2×10^{9}	Fry et al., 1938	5×10^{6}	Fry et al., 1938

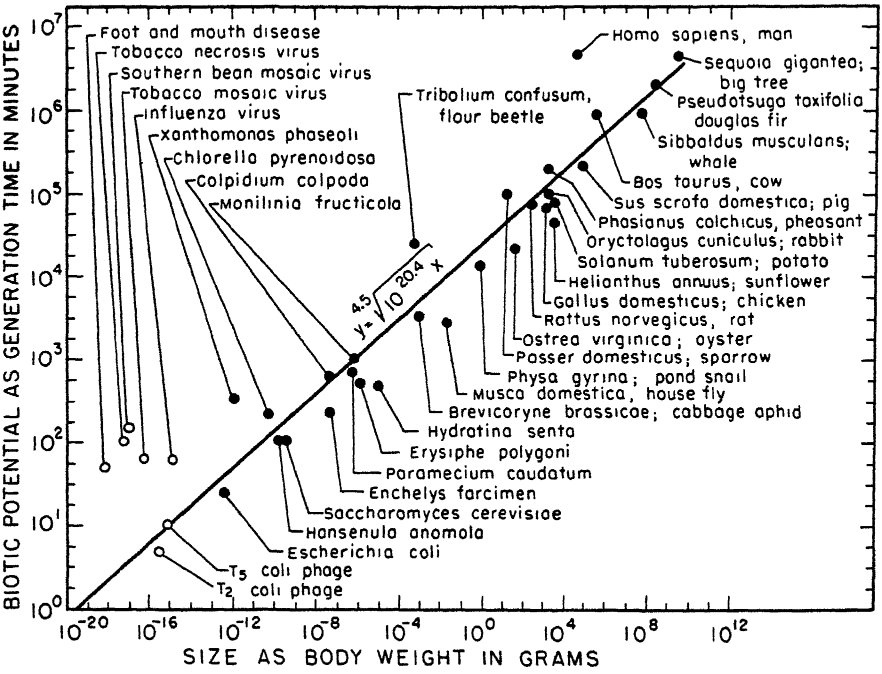

FIGURE 1. Relation of body weight to rate of reproduction.

the increase in population would be $\frac{4500}{2}$ or 2250 fold (logarithm of 2250 = 3.353) and the generation time would be $\frac{154 \times 24 \times 60 \times 0.301}{3.353} = 1.99 \times 10^4$. Because of the uncertainty and variability of values, size and generation times are given to only the nearest digit (e.g. 2320 would be given as 2×10^3).

Representative organisms from various taxonomic groups were chosen on the basis of available data, even though these data were in many cases somewhat inadequate. In several cases it was easier for me to weigh or measure a representative or group of individuals than to look for published information on body size. For the published data secondary sources were sometimes used. Reproduction rates for the 17-year locust (*Magicicada septendicim*) and the tuberculosis bacterium (*Mycobacterium tuberculosis*) were intentionally omitted because it is believed that these organisms reproduce at a much slower rate than is typical of the taxonomic groups in which they occur.

RESULTS

Results for 28 species of conventional organisms and for seven viruses are given in table 1 and figure 1. There is a fairly progressive increase in generation time with increase in body weight through many taxonomic groups of plants and animals. That this is a straight line relation cannot be established from the limited data, but it seems a useful hypothesis. If so, the average generation time for the 28 species of conventional organisms including bacteria, algae, fungi protozoa, insects, snails, birds, mammals, herbs and trees is about $\sqrt[4.5]{10^{20.4}X}$ when X is body weight in grams. The seven viruses as a group fit fairly well into this relation of generation time to body weight for conventional organisms.

The two bacteria and seven viruses listed illustrate what may be significantly greater generation times for tissue-invading than for free-living forms. Data for *E. coli* (16 minutes generation time) is for free living forms in broth, while data for *X. phaseoli* (260 minutes generation time) is for infections in bean leaves. Several other free-living forms could be substituted for *E. coli* and several other tissue-invading forms could be substituted for *X. phaseoli*, or the generation time of *X. phaseoli* could be compared as a free living form (Mason, 1935) and as a tissue invading form (Allington and Chamberlain, 1949), with the same trend in the data. With the viruses, while all are intracellular, the phages are similar to free living forms in that they are free in the medium after the burst of each host cell, and their generation times appear significantly lower than the generation times of the tissue-invading forms. If the values presented for tissue-invading forms of bacteria and viruses could be corrected by a factor by which the tissue-invading forms and free-living forms differ, the values for tissue-invading bacteria and viruses would be closer to the value for the other conventional organisms. It is also likely that if generation times for tissue-invading viruses could be determined within single cells, the times would be much less.

It may also be noteworthy that the rate of increase has been more intensively studied with the bacteriophages than with tissue-invading viruses. As the tissue-invading forms are studied more intensively, it is likely that in the future as in the past (Yarwood, 1952) the generation times for these viruses will be found to be considerably less than the early or present values. As these values for tissue-invading viruses are reduced with further research, the body weight: generation time relation for viruses will probably fit more closely the values for conventional organisms.

SUMMARY

The generation time (time for doubling of the population) under optimum conditions for 28 conventional organisms ranging from bacteria to redwood trees increased at a fairly regular rate with increase in body weight. The average generation time was about $\sqrt[4.5]{10^{20.4}X}$ or $X^{0.227} \times 10^{4.4}$ minutes when X was the body weight in grams. The generation times of two bacteriophages were very close to this value, but the generation times for five tissue-invading viruses were much greater. On this basis and on the basis of plausible corrections for tissue-invading viruses, it is believed that the relation of body weight to rate of reproduction is basically the same for viruses as for conventional organisms.

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STUDIES ON MATERNAL RETRIEVING IN RATS: II. EFFECTS OF PRACTICE AND PREVIOUS PARTURITIONS[1]

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Many types of behavior are executed imperfectly or incompletely at the time of their first occurrence, and become perfected and efficient only as a result of practice. Some other responses appear in well-developed and effective form the first time they occur. Thus the female rat that has never built nests or born young will typically construct a nest toward the termination of her first pregnancy, and will deliver, clean, and nurse her first litter successfully. This conclusion is substantiated by the work of several investigators (Weisner and Sheard, 1933; Beach, 1937; Stone, 1938), and the biological adequacy of the primiparous mother seems well established. At the same time, there remains the possibility that maternal efficiency might improve as a result of the experience gained in successive parturitions, and care of the young might become more effective in the course of a single lactation period. The present experiment was conducted to discover whether either or both of these possibilities are reflected in fact.

SUBJECTS AND METHODS

Subjects were 19 multiparous and 18 primiparous rats of a pigmented strain. Each animal was placed in a special observation cage approximately five days prepartum and remained there until maternal tests were completed. The wooden cages were 3' × 3' × 1' and were divided by a central partition pierced by a small doorway. Connected with the outer wall of each half of the cage was a covered release box (6" × 4" × 4") separated from the main cage by a vertically sliding door. Strips of paper towelling suspended from the cage walls served as nesting material. The observation cages were situated in a quiet, darkened room, and each one was illuminated by a 40w bulb.

Each cage was inspected several times a day, and within a few hours after parturition the size of the litter was recorded. In addition notes were made of the number of young which were well or poorly cleaned. On the day of parturition the nest was photographed and rated on a five-point scale. At regular intervals postpartum the female's retrieving behavior was tested. The mother was gently removed from the cage and confined in the release box farthest from her nest. Young were picked up with forceps and placed at predetermined locations on the cage floor. The door to the release box was raised and a timer was started when the female entered the observation cage.

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[1] This study was supported in part by a grant from the Committee for Research in Problems of Sex, National Research Council. The experimental observations were carried out in the Department of Animal Behavior at the American Museum of Natural History.

In all retrieving tests records were made of the number of young returned to the nest at six standard intervals. These were .5, 1, 5, 10, 30, 60 minutes from the time the female left the release box and entered the main cage. In some of the tests notes were made of the time at which each individual pup was retrieved. A special method of recording the mother's movements about the cage was utilized upon several occasions. The cage floor was marked off into 36 equal, numbered squares, and as the female walked about, the number of each square she entered was tabulated. All retrieving tests lasted until the last pup had been retrieved or until 60 minutes had elapsed.

Ten of the primiparous females (Group I) were given a minimum of five daily retrieving tests beginning on the day of parturition. Six of these animals were also tested on the sixth day, and four on the seventh day postpartum. The remaining eight primiparae (Group II) were given only one retrieving test. For two females this occurred on the day of parturition. Three additional mothers were removed from the cage and handled briefly on the first two days, just as if retrieving tests were being conducted, and then tested for the first time three days postpartum. The final three cases were handled daily for six days postpartum, and then given the retrieving test on the seventh day after giving birth.

Ten of the multiparous rats (Group III) had previously reared litters in small breeding cages but had never been tested for nest-building or retrieving. This group was given a minimum of five daily retrieving tests beginning on the day of parturition as were Group I. The other nine multiparae (Group IV) had previously littered in the special observation cages, and had been observed in four to eight formal retrieving tests at that time. On the day of the second parturition, they were given retrieving tests and the results compared with their previous records.

RESULTS AND DISCUSSION

Parturition and retrieving behavior of multiparous and primiparous females. Comparisons of the parturient behavior of females in Groups I and

TABLE 1

VARIOUS MEASURES OF MATERNAL BEHAVIOR FOR TEN MULTIPAROUS AND TEN PRIMIPAROUS FEMALES

Group mean	Primiparous Group I	Multiparous Group III
Number of pups born	8.3	8.8
Number of pups poorly cleaned	0.5	0.7
Number of pups out of nest	0.0	0.3
Median grade of nest		
Day before parturition	3.5	1.0
Day after parturition	3.5	1.5

TABLE 2

AVERAGE CUMULATIVE NUMBER OF PUPS RETRIEVED AT VARIOUS INTERVALS FOR 10 PRIMIPAROUS (P) AND 10 MULTIPAROUS (M) FEMALES ON FIVE SUCCESSIVE DAYS AFTER PARTURITION

Time intervals in minutes	Day 1		Day 2		Day 3		Day 4		Day 5	
of test	P	M	P	M	P	M	P	M	P	M
0.5	0.8	0.4	1.1	1.3	2.7	1.9	2.9	2.8	2.8	2.8
1	1.8	1.3	2.0	2.7	4.7	3.3	4.9	4.6	4.8	4.2
5	5.3	3.9	5.0	5.2	5.9	5.2	5.9	5.8	5.9	5.5
10	5.4	5.0	5.7	6.0	5.9	5.3	5.9	6.0	5.9	5.6
30	5.8	5.5	6.0	6.0	6.0	5.5	5.9	6.0	5.9	6.0
60	6.0	5.5	6.0	6.0	6.0	5.9	5.9	6.0	5.9	6.0

III indicate that the experience of bearing and rearing one litter has no effect upon nest-building or caring for the young at the termination of a second pregnancy. The relevant comparisons appear in table 1. None of the differences are statistically reliable.

The primiparae of Group I had no experience in retrieving young. The multiparae of Group III had a somewhat comparable history in that their previous litters had been reared in small cages where necessity or opportunity for retrieving was very limited. The retrieving behavior of these two

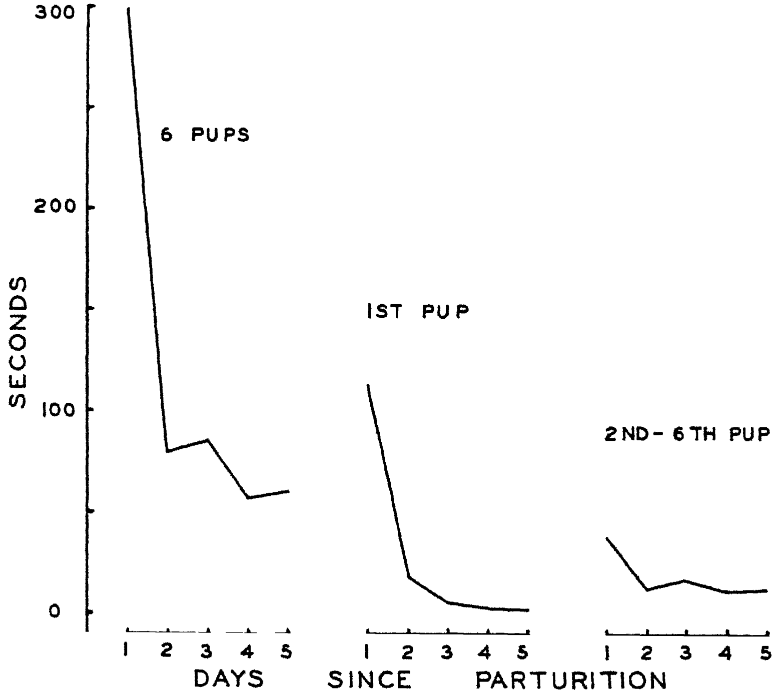

FIGURE 1. Median total time to retrieve all six pups, median time to retrieve the first pup only, and the average of the median times to retrieve the 2nd, 3rd, 4th, 5th, and 6th pups, on five successive days following parturition.

types of females is compared in table 2, and it is evident that no appreciable differences existed.

Day-to-day changes in retrieving. Table 2 does show an improvement in retrieving performance on successive daily tests, and the rate of change is comparable for multiparous and primiparous females. Combining the averages for the two groups, on the day of parturition an average of 1.6 pups were retrieved during the first minute of the test. By the fifth day postpartum performance had improved to the point that an average of 4.5 young were retrieved to the nest in the first 60 seconds. Comparing the number of pups retrieved during the initial 60 seconds of the first and fifth tests, increases in this score were found for 18 females and there was no change in the remaining two cases. This group increase in rate of retrieving is therefore statistically reliable by the sign test at the one per cent level of confidence.

Figure 1 reveals one source of the changes responsible for increases in the speed of retrieving. This figure is based on records of the seven animals for which the retrieving time of each pup was recorded. It is apparent that most of the day-to-day acceleration in total retrieving was due to a decrease in the delay preceding retrieving of the first pup. No appreciable change occurred in the time taken to complete the retrieving of the remainder of the young once retrieving had started. Furthermore, most of the increase in speed of retrieving the first pup took place on the second day postpartum.

In the case of eight animals (4 from Group I and 4 from Group II) records were kept of the female's movements about the cage during the retrieving test. The number of squares entered from the beginning to the end of the retrieving test tended to decrease on successive days. On the first day postpartum, an average of 110 squares was entered. On succeeding days this value was 82, 85, 73, and 63. The progressive decrease in the number of squares entered suggests a second sort of change responsible for increased speed of retrieving, namely that females tended to engage in less exploration either before beginning to retrieve or during intervals between the retrieving of successive pups.

Retrieving in unpracticed primiparae. Changes in the speed of retrieving could be due to factors other than practice. Firstly, females might become progressively stronger and more active for several days after giving birth. Secondly, the fact that young increase in size and motility might make them more prominent stimulus objects to be approached and retrieved. Thirdly, adaptation to handling by the experimenter could account for considerable improvement, particularly in the speed with which retrieving was begun. For these reasons, the results of tests on primiparous rats in Group II are important.

These animals were given their first retrieving test on the first, third, or seventh day postpartum, and were handled daily up to the day of testing. The results showed no significant difference in the retrieving behavior of

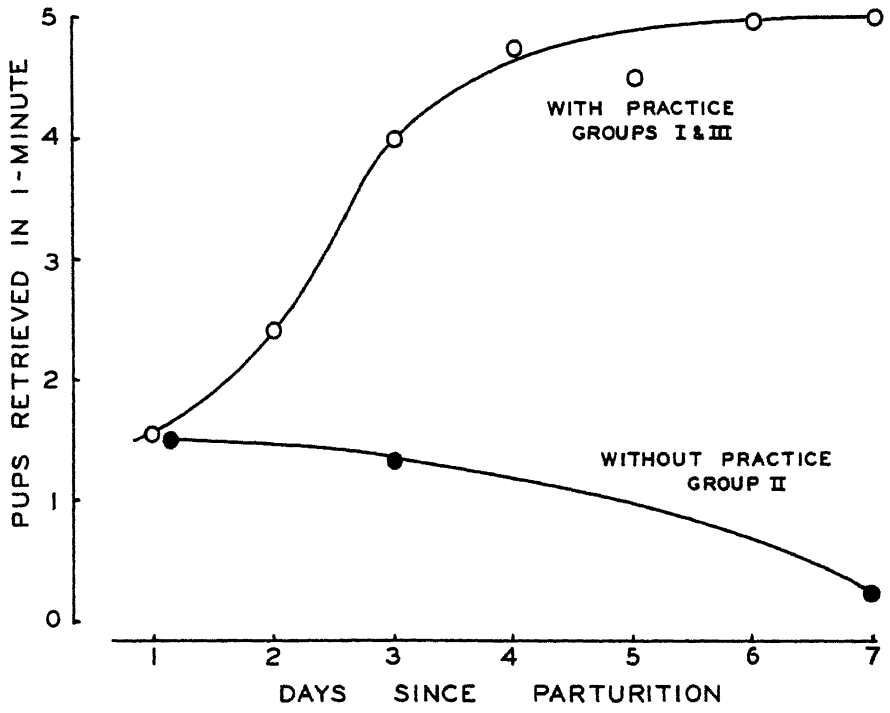

FIGURE 2. Retrieving with and without practice as a function of days since parturition.

females receiving their first tests at these three intervals after parturition. The average number of pups retrieved within the first minute is shown in figure 2 where the scores of animals in Group II are compared with the combined scores of Groups I and III. Differences between the performance of mothers with practice and without practice are statistically reliable, despite the small number of animals in the three subdivisions of Group II. On both the third and seventh days, the P value is less than .03 by the Mann-Whitney U test. Apparently, improvement shown during successive days of

TABLE 3

CUMULATIVE SCORES SHOWING AVERAGE NUMBER OF PUPS RETRIEVED AT SUCCESSIVE INTERVALS ON THE FIRST DAY POSTPARTUM

Min. from start of test	Group I First pregnancy	Group IV First pregnancy	Group IV Second pregnancy
0.5	0.8	0.4	0.7
1.0	1.8	0.9	1.3
5.0	5.3	4.4	3.4
10.0	5.4	5.4	3.6
30.0	5.8	5.4	4.1
60.0	6.0	5.7	5.1

retrieving cannot be referred solely to changes in the young, to the time elapsed since parturition, or to effects of handling on successive days.

Retrieving in successive parturitions. Females in Group IV were tested for retrieving in two successive lactation periods. Their performance did not change significantly from one pregnancy to the next. The average number of young retrieved at the end of each of the standard time intervals is shown in table 3 where the performance of Group I is included for purposes of comparison. It is evident that the performance of Group IV during the second pregnancy was not superior to that of the same females during their first lactation period or to the retrieving of the primiparous mothers in Group I. As measured by the number of pups retrieved during the first minute of the test, 6 of the nine subjects in Group IV showed poorer retrieving following the second pregnancy, three improved and the final case showed no change in the behavior.

Two major conclusions are indicated by the results of this study. First, the retrieving behavior of lactating female rats improves if they are tested once a day starting on the day of parturition. Part of the improvement consists of a shortening of the delay preceding the retrieving of the first pup. Another feature is a reduction in the amount of territory covered in the course of retrieving the entire litter. Reduction of the initial delay is most pronounced between the first and second test. Decrease in total distance travelled occurs progressively over a series of five daily tests.

The second major conclusion is that the maternal behavior of primiparous and multiparous mothers does not differ significantly. Experience in giving birth, nest-building and retrieving during one pregnancy and lactation period has no effect upon maternal performance in a second reproductive episode.

SUMMARY

The maternal behavior of 19 multiparous and 18 primiparous rats was compared. Females that had previously born and reared litters did not differ significantly from primiparous animals in prepartum nest-building or cleaning of the young at the time of parturition. Some multiparous individuals had been given special retrieving tests during their first lactation period. Following a second pregnancy the retrieving behavior of these individuals differed in no appreciable way from that of other females which had just delivered their first litter.

When multiparous and primiparous females were observed in a series of daily retrieving tests their behavior improved, and the course of improvement was the same for both groups. Various controls indicated that this improvement was not due to changing qualities of the growing young. Increased efficiency of retrieving could be traced to two kinds of change. The first consisted of a rather sudden decrease in the delay preceding the initiation of retrieving once a test had begun. This period of delay was marked in the first test but was greatly reduced on the second day and on

all days thereafter. A second change involved a reduction in the amount of locomotor activity associated with retrieving. On successive tests the females tended to proceed more and more directly to the scattered pups and to retrieve them with a minimum of interpolated exploration of the cage.

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MUTATIONS AT A SINGLE LOCUS IN THE WASP MORMONIELLA

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In the wasp Mormoniella with haploid males from unfertilized eggs, diploid females from fertilized, many recessive eye-color mutants have been found ranging from dark red approaching the dominant wild-type brown through bright reds, orange, peach and tinged to oyster white (colorless). Irradiation of unmated females induces mutations in the eggs resulting in mutant sons in frequencies as high as one per cent of survivors. Spontaneous mutation is rare in pure stocks but frequently occurs in pedigrees after crossing different stocks. The majority of eye-color mutants occur at a certain locus, *R*, but many are scattered, segregating independently or showing linkage.

Several studies have already been made by this (first) method of breeding unmated irradiated females and dose-action curves have been obtained for X-rays and for fast and slow neutrons (Ray and Whiting, 1954, Kayhart, in press). The visible eye-color mutations resulting are all viable because recessive lethals are filtered out by male haploidy.

In order to recover recessive lethals which have visible effects in compounds and to restrict observations to the *R* locus alone, a second method has been devised making use of an *R*-locus recessive gene called peach-333.5, *pe*-333.5. Other genes causing peach eye color occur both at the *R* locus and at other loci, but peach-333.5 is the only eye-color tester gene employed in the present discussion, where it will be called simply peach or *pe*.

Because peach is an *R*-locus recessive gene any mutant-type daughters will indicate an *R*-locus mutation induced in the treated wild-type egg. The irradiated wild-type females are mated immediately after treatment to untreated peach-eyed purple-bodied males. Purple is linked with *R* giving ten per cent crossovers. Most of the offspring from this cross will be wild type, the females heterozygous, $\frac{+\quad +}{pe\quad pu}$, the males azygous, $\underline{+\quad +}$. A scarlet-eyed female, $\frac{st\quad +}{pe\quad pu}$, would indicate that a mutation was induced

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allelic with peach. A mutation to peach would result in a peach female, $\frac{pe\ \ +}{pe\ \ pu}$, or a peach male, $\frac{pe\ \ +}{\ }$.

In study of mutations it is important to consider other exceptional types. If a peach purple female, $\frac{pe\ \ pu}{pe\ \ pu}$, or male, $\frac{pe\ \ pu}{\ }$, should appear it would be highly unlikely that this would be due to mutation at the two loci. A large deficiency might result in a peach purple female, $\frac{-Df-}{pe\ \ pu}$, but androgenesis would be a more probable explanation. Androgenesis, development from the sperm nucleus alone without fusion with egg pronucleus, has been reported after X-radiation of females in Mormoniella (Friedler and Ray, 1951). In the wasp Habrobracon androgenesis occurs when the egg pronucleus is inactivated by irradiation (Whiting, Anna R., 1948). An androgenetic daughter or son is not the offspring of the female that lays the egg. Shell, yolk and egg cytoplasm are but the pre-hatching food and environment, just as host pupa and puparium or caterpillar are the post-hatching food and environment of this motherless anomaly. Its "brothers" are not its brothers for they are impaternate while it is immaternate.

The bright eye colors have been selected for study of mutation rates because these are so strikingly different from wild-type brown that they cannot be missed. It may also be safely assumed that they are true mutants since all that have been tested have proved to be such. With good light and against a dark background, dark red eye colors are easily identified but these grade into reddish brown and some of them are fluctuations of wild type. They are, therefore, not included in calculating mutation rates. The present paper reports results obtained by the second method. Wild-type females of Woods Hole stock were X-rayed and then crossed to peach purple males.

PRESENTATION OF DATA

Experiment 1. The first experiment was carried out by the junior author. Offspring per female set were 44.8 for the 98 control females, 5.6 for the 166 females treated with 3000 r and 3.5 for the 1267 females treated with 3500 r. No androgenetic offspring were produced. No mutants occurred in the controls (1798 females, 2593 males). From treated females there were 22 bright eye-color mutants (4 tomato, 11 scarlet, 4 peach, 3 oyster) among the 1807 males and 36 (1 tomato, 23 scarlet, 3 peach, 9 pale peach) among the 3599 females. Of the 14 male mutants successfully mated, 7 (4 tomato, 2 scarlet, 1 peach) sired wild-type daughters suggesting loci other than *R*, and 7 (4 scarlet, 3 oyster) gave mutant-type daughters indicating *R*.

The pale peach female mutants are oyster-peach compounds, $\frac{oy\ \ +}{pe\ \ pu}$.

dicating mutation to oyster in the treated eggs. Four were bred producing many oyster and peach purple (parentals), few oyster purple and peach (crossovers) sons.

Of the nine scarlet mutant females that were bred, six, $\frac{st\ \ +}{pe\ \ pu}$, produced many scarlet and peach purple (parentals), few scarlet purple and peach (crossovers) sons. These six mutations were scarlet viables.

The other three scarlet mutant females tested produced no scarlet sons but only peach (peach purple parentals, peach crossovers). These were scarlet lethal mutants because the scarlet gene here has a recessive lethal effect. A scarlet lethal female, $\frac{stl\ \ +}{pe\ \ pu}$, crossed to a peach purple male produces daughters:- scarlet, $\frac{stl\ \ +}{pe\ \ pu}$, and peach purple, $\frac{pe\ \ pu}{pe\ \ pu}$, many (parentals) and scarlet purple, $\frac{stl\ \ pu}{pe\ \ pu}$, and peach, $\frac{pe\ \ +}{pe\ \ pu}$, few (crossovers). From her unfertilized eggs, as from the eggs of an unmated female, there are produced only peach purple, $\underline{pe\ \ pu}$ (parentals) and peach, $\underline{pe\ \ \ +}$ (crossovers) males, because those carrying scarlet lethal, $\underline{stl\ \ +}$ and $\underline{stl\ \ pu}$, are inviable.

Rate of mutation at the *R* locus alone including recessive lethals, 2.93 × 10^{-6} per r, as shown in the females, is not significantly lower than rate at all loci together if lethals are not included, 3.58 × 10^{-6} per r, as shown by the males. The identification of haplo-inviable mutations in the *R* locus when the former method of testing for mutation rate was used demonstrates the value of this method.

Experiment 2. The senior author carried out a second experiment with the same stocks. Wild-type females, 2850 in number, were X-rayed (3500 r) and crossed with peach purple males. The purpose of this experiment was not to determine mutation rate, but rather to obtain mutants for genetic analysis. Therefore number of offspring was not counted. However, a rough estimate was made. This estimate, 10,000 from 2850 mothers, proved very close to the average number, 3.5, of counted offspring per female X-rayed with 3500 r in the earlier experiment by the junior author.

Among the males there was one peach-eyed. He was probably androgenetic rather than a mutant since he had the paternal purple body color. Among the females there were seven peach purple, androgenetic. There appeared 19 bright eye-color mutants (2 tomato, 1 vermilion, 7 scarlet, 2 peach, 7 oyster) among the males (viables at any eye-color locus) and 10 (2 pale peach, 7 scarlet, 1 dahlia) among the females (viables and recessive lethals at *R* locus only).

Daughters were obtained from seven of the nineteen bright-eyed mutant males,- the single vermilion, 2 scarlet, 1 peach and 3 oyster. Tests made by crossing these males with bright eyes to peach purple females showed peach purple sons as expected from unfertilized eggs. Because none of the daughters was wild type, these seven mutations occurred at the *R* locus. Daughters of the peach mutant were exactly like their peach purple brothers in eye color and in F_2 the mutant peach could not be separated from peach-333.5. In contrast, the pale peach daughters of each of the three oyster mutants were distinctly lighter than their peach purple brothers and the expected difference was noted among the F_2 females. The vermilion and scarlet males sired vermilion and scarlet daughters respectively. F_2 crossovers from the vermilion, scarlet and oyster mutant males totalled 90 among the 1016 counted, 8.86 per cent.

Six of the seven androgenetic females were tested. Four of these produced 259 wild-type daughters, $\frac{pe\ \ pu}{+\ \ \ +}$, and 270 peach purple sons. They had mated with the wild-type males from the treated females. Offspring from 58 daughters of these androgenetic females showed peach-purple linkage as expected.

The fifth androgenetic peach purple female produced peach purple only, 26 females and 7 males. It is highly unlikely that this androgenetic female should have mated with an androgenetic male (only one was noted in the mass culture). A mating with a son of a treated female, wild-type but mosaic for a peach-purple deficiency, would have produced peach purple daughters, $\frac{pe\ \ pu}{-\ Df\ -}$. Thirteen were tested producing males only, many peach purple.

The sixth androgenetic peach purple female produced 68 wild-type and 6 pale peach, non-purple daughters and 41 peach purple sons. Fifteen of the wild-type daughters, $\frac{pe\ \ pu}{+\ \ \ +}$, were tested showing peach-purple linkage as expected. The six pale peach daughters were bred and proved to be peach-oyster compounds, $\frac{pe\ \ pu}{oy\ \ +}$. This androgenetic female had probably mated with a male mosaic for oyster, the son of an X-rayed female.

The two pale peach mutant females were compound for oyster mutations induced in the treated eggs. Purple linkage was shown in their progeny.

Four of the six scarlet mutant females tested showed purple linkage and normal viability of scarlet in their sons but the other two were scarlet-lethal producing no scarlet among their 109 sons. From their wild-type daughters scarlet-lethal females were derived, compound for peach and producing only peach sons. Linkage with purple was shown, totalling 463 parentals, 49 crossovers, 9.57 per cent. A scarlet lethal stock descended from one of these mutants was bred. Counts for eye color of offspring of scarlet mothers totalled females,- scarlet 1615, peach 1594 and males,- peach 4107, oyster mutant one.

The mutant female with dark red eyes, dahlia, proved to be dahlia-semilethal, *dasl*. Males with this gene are highly inviable and most of those that do mature are much deformed and separable for body color with difficulty. Sons of the mutant showed purple linkage of *dasl* giving an excess of peach purple. The fifteen wild-type daughters tested included all four expected types,- $7\,\frac{pe\ \ pu}{+\ \ \ +}$, $1\,\frac{pe\ \ +}{+\ \ \ +}$, $1\,\frac{dasl\ \ pu}{+\ \ \ \ +}$ (which produced males,- wild type 66, purple 13), $6\,\frac{dasl\ \ +}{+\ \ \ \ +}$ (which produced females,- wild type 35, dahlia 25 and males,- wild type 467, dahlia 3.

Two of the 25 F_2 dahlia females produced males,- dahlia 35, dahlia purple 8, dahlia (purple?) 17, peach (mostly purple) 503, oyster purple mutant 1. Their $\frac{dasl\ \ +}{+\ \ \ \ +}$ mother had therefore mated with a peach purple brother.

Some of the dahlia males were relatively normal and it was possible to get matings with peach purple females. There resulted 321 dahlia females and 578 peach purple males. These dahlia females, $\frac{pe\ \ pu}{dasl\ \ +}$, produced males,- dahlia 91, dahlia purple 19, dahlia (purple?) 56, peach 143, peach purple 710. Males with dahlia eyes are far outnumbered by their peach-eyed brothers showing that the semilethal effect was still associated with the dahlia eye color. These relatively normal appearing dahlia males were therefore fluctuants of dahlia-semilethal rather than crossovers between dahlia and a closely linked semilethal.

Grandsons of selected relatively normal dahlia males were 166 dahlia, 853 peach, giving 19.46 per cent relative viability. In unselected stock there were 68 dahlia males with 1073 brothers, 6.34 per cent relative viability. The difference is very highly significant suggesting factors compensating for the semilethal influence of *dasl*.

Daughters of dahlia females crossed with their peach brothers total dahlia 168, peach 178, showing that the semilethal effect of *dasl* is recessive.

Crossovers between *dasl* and *pu* total 188, 17 per cent, among 1108 males that were separable for body color.

Mutants were found among the descendents of the androgenetic and mutant females of this experiment.

In addition to the deficiency suggested to explain the peach purple daughters of androgenetic female number 5 and the oyster found in six of the 74 daughters of androgenetic female number 6, there were ten unexpected eye-color types.

A mosaic female with a scarlet spot in her right eye, due either to a second mutation or to egg binuclearity, occurred among the wild-type daughters of one of the scarlet mutants. This mosaic female bred like her wild-type sisters, heterozygous for peach and purple. Among her sons were three oyster purple, spontaneous mutants probably from peach purple.

The six pale peach daughters of peach purple androgenetic female number 6 produced sons,- peach purple 297, oyster 259, peach 46, oyster purple 41 and daughters,- pale peach and peach purple many, peach and pale peach purple few, and a single scarlet purple. This spontaneous mutant bred as compound for peach purple mated with a peach purple brother. The scarlet gene acted as a dominant to oyster- 250.6 and showed puprle linkage. The oyster- 250.6 stock was derived from a spontaneous *R*-locus mutant male.

Three oyster purple males occurred among the 14 peach and 92 peach purple sons of one of the scarlet-lethal mutants. One of these bred to scarlet-DR females proved recessive siring scarlet daughters which showed purple linkage in their offspring. The scarlet-DR stock was derived from the first X-ray *R*-locus scarlet mutation.

A peach purple female and a dahlia-semilethal female in F_2 from the dahlia-semilethal mutant female each produced a single oyster purple mutant male among their numerous peach purple sons. Each of these tested with scarlet-DR females proved recessive with purple linkage in F_2.

DISCUSSION

Dose-action curves have hitherto, for the most part, been based on lethals, either dominant or recessive. Lethals include a very great diversity of conditions cytogenetically, ranging from gross structural aberrations to submicroscopic alterations. Two-hit curves are expected from the former, one-hit from the latter.

It has generally been considered that rates for visible mutations should form a one-hit curve because visibles are regarded as point mutations. There is evidence that visibles affecting eye color induced in the eggs of Mormoniella by treatment with X-rays and with fast and slow neutrons do not form a one-hit curve but that the rate is increased disproportionately at higher doses (Kayhart, in press). The cytogenetic significance of this fact is not understood. These rates have been obtained by the first method mentioned in this paper, from counts of haploid males developed from treated eggs. Male haploidy has filtered out all lethals and the curves are based on survivors with all mutable eye color loci included.

Genetically considered, a "point" is a mendelizing unit which cytologically may be anything from an entire chromosome to a submicroscopic region of a chromonema. Points differ among themselves in mutability so that, while a curve based on many points may be repeated in different experiments, its cytogenetic significance is little if any better understandable than a curve for lethals.

A dose-action curve might be based on mutation rate at a single point, provided mutation frequency were high enough to make this possible. Data given in Experiment 1 of this paper indicate this to be the case for the *R*-locus of Mormoniella. Total rate for *R* alone is not statistically lower than rate for viables at all loci and unpublished data indicate that rate for viables at *R* actually exceeds rate for viables at all other loci taken together. This may be because the *R* locus is physically more extensive than other eye-color loci. Some of the compounds of *R* alleles exhibit complementarity, "pseudoallelism," and it is considered that there are two mutable color elements involved (Whiting, P. W., 1951, 1954). Rate for viables at *R* cannot be directly determined from observation of males. Many tests of male mutants fail so that rate for *R* must be determined by extrapolation from the proportion of *R* mutants among those successfully tested.

The second method of collecting data for study of mutation rate should prove to be more satisfactory in that all visibles are recovered including those with recessive lethal effect. Moreover, the chance of establishing and maintaining a stock with each mutant gene is very high because most females will produce offspring. Thus it is possible not only to determine total rate at a single locus, but to identify almost every mutation with respect to its genotype as well as its phenotype. A phenotypically scarlet for example may be a viable, a recessive lethal or a female-sterile. It may affect one gene element or more than one. It may be a mutation from the wild-type allele or from any one of the mutant alleles. Scarlet mutants have already been obtained from several of these alleles, not only the colored but also oyster white.

Spontaneous mutations at the *R* locus occur with relatively high frequency in miscellaneous crosses involving different *R*-locus alleles. Their rate has not been determined because it was requisite to record only classes of offspring produced. For example, a mutant male crossed to a tester female, compound for two alleles, sires two types of daughters in equal numbers. A sample of each of these daughters produces two classes of sons in equal numbers. Establishment of the 1:1 ratio would be, in each case, both laborious and unneccessary. It can be observed at a glance that the types are at least approximately equal. Of more importance is the recording of the two colors produced. Then there may appear one or a few mutants quite distinct in color from their numerous sibs. These have in general proved to be in the *R* locus.

Data presented in this paper are but a small sample of those already obtained. It has become obvious that spontaneous rate in mixed pedigrees is higher than in pure stocks, although the former cannot be calculated because of incompleteness of counts. Studies are in progress in which pairs of stocks are being crossed and F_2 males counted. These already indicate significant differences among themselves and from rates for pure stocks. There is need for further testing of many different *R*-locus types to give a better understanding of this complicated series of genes.

SUMMARY

Induced mutations to bright eye colors in Mormoniella have hitherto been obtained by observation of sons of irradiated unmated females. Mutation rates up to one per cent of survivors thus obtained are based on viable changes at all eye-color loci. It has been shown by breeding tests of the mutant males that the majority of these mutations occur at a single locus called *R*.

The present paper presents a second method for securing mutations. X-rayed wild-type females are mated to untreated males with peach eyes, a

recessive at the *R* locus. Daughters produced are, therefore, wild-type unless a mutation has occurred at this locus. By this method mutation rate can be studied at a single locus and mutant types with recessive lethal effect are recovered as well as viables. It has been shown that, within the limits of the data, this total mutation rate at the single locus *R* is high being not significantly less than the rate for viables at all loci including *R*.

Androgenetic offspring, both males and females, are produced following irradiation of eggs.

In pedigrees following genetic tests of *R*-locus mutants, further mutations occur at this locus. Their rate has not been determined but it appears to be much higher than spontaneous rate in pure stocks.

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THE EFFECT OF OXYGEN CONCENTRATION ON THE DOSE-ACTION SURVIVAL CURVES OBTAINED FOR HABROBRACON EGGS IRRADIATED DURING MEIOTIC PROPHASE AND METAPHASE.*

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Thoday and Read (1947) found that bean root cells X-rayed in the absence of oxygen contained a lower proportion of chromosomal aberrations than those irradiated in the presence of oxygen. Later studies (Giles and Riley, 1949; Hayden and Smith, 1949; and Baker and Sgourakis, 1950) have shown that most types of genetic changes caused by X-rays vary with the oxygen concentration surrounding the cells at the time of irradiation.

Giles and Riley (1950) pointed out that the dependence of the genetic effects of X-rays upon oxygen concentration could be the result of an augmentation of the initial effect of the X-ray quanta or could result from inhibition by oxygen of the restitution of X-ray damage once it had occurred. They found that oxygen increased the percentage of aberrations only when present at the time of irradiation and had no effect before or after irradiation. From this they concluded that oxygen probably acted by augmenting the initial damage rather than by preventing restitution of damage.

Schwartz (1952) studied the effect of oxygen concentration on the production by X-rays of mosaic and non-mosaic phenotypes in corn. He assumed that a kernel mosaic for two closely linked markers was the result of a terminal deletion in the X-rayed pollen and considered a completely recessive kernel to be the result of an interstitial deletion in the X-rayed pollen. Irradiation in the absence of oxygen caused a greater percentage decrease in mosaic kernels than in recessive kernels, and Schwartz concluded from this that oxygen prevented the restitution of chromosomal damage in his material.

Whiting (1945a) found that X-rays cause a far higher percentage of lethality in Habrobracon eggs irradiated during meiotic metaphase-I than they cause in those eggs irradiated during meiotic prophase I with the same doses. Her cytological studies (1945b) showed that almost all lethality induced in prophase-I or metaphase 1 eggs resulted from the loss of parts of chromosomes during meiosis following irradiation. She suggested that meiotic metaphase eggs were considerably more sensitive to X-rays than

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meiotic prophase eggs because of a large difference in the ability of the chromosomes in each of these two stages to recover from X-ray damage. Metaphase chromosomes are stretched on the spindle and, according to her interpretation, X-ray damage probably results in the deletion of a terminal segment of the chromosome with little chance of restitution. In contrast to metaphase chromosomes, prophase chromosomes do not appear to be under tension and restitution of X-ray damage seems more probable.

On the basis of Whiting's findings, it seems likely that experiments with Habrobracon eggs can provide some information on the role of oxygen in augmenting the effects of X-ray damage to chromosomes. If oxygen prevents the restitution of chromosomal damage, oxygen concentration will have a very small effect on the percentage of lethality induced in metaphase eggs in which the restitution of chromosomal damage is relatively rare. On the other hand, prophase eggs irradiated in higher oxygen concentrations will show a higher percentage of lethality than those irradiated in the absence of oxygen because Whiting's data showing that prophase I eggs are relatively insensitive to X-rays indicate that damage to prophase chromosomes can be restored. A comparison of the effectiveness of oxygen in augmenting radiation damage in each of the two stages should provide evidence which supports the restitution hypothesis of Schwartz or the breakage hypothesis of Giles and Riley. This comparison has been made in the present study for various X-ray doses and the data obtained favor the breakage hypothesis.

In the series of experiments described below, the relationship of X-ray dosage to the induction of total lethals was determined in the presence ol nitrogen, air, and oxygen. These lethality values were determined for unfertilized eggs of Habrobracon irradiated both in the prophase and metaphase of the first meiotic division. Simple techniques for studying the effects of X-rays on different meiotic stages have been worked out for this organism by Whiting (1945a).

EXPERIMENTAL RESULTS

Eggs in meiotic metaphase-I were irradiated with 396r, 1,120r, 1,493r, 1,862r, and 2,450r. At each of these dosages up to 1,862r, three groups of animals were irradiated in each experiment; one in nitrogen, one in air, and one in oxygen. Beyond 1,862r animals were X-rayed only in nitrogen because the results of the 1,862r experiment showed that 100 per cent lethality was induced in eggs X-rayed in air or oxygen above that dosage.

Results of the metaphase experiments are given in table 1. When irradiated in nitrogen the percentage of eggs hatching decreased progressively from 79.7 ± 4.0 per cent when irradiated with 396r to 8.0 ± 3.4 per cent of eggs irradiated with 2,450r. In air the percentage of eggs hatching at 396r was 49.2 ± 5.4 per cent and this dropped progressively until 0.2 ± 0.4 per cent were found to hatch at 1,862r. When irradiated in oxygen the percentage of eggs hatching was 40.6 ± 5.4 per cent at 396r and declined to 4.0 ±

TABLE 1

HATCHING RATIOS OF HABROBRACON EGGS IRRADIATED IN NITROGEN, AIR AND OXYGEN DURING MEIOTIC METAPHASE I. FRACTIONS REPRESENT EGGS HATCHING OVER TOTAL EGGS COUNTED AND ARE SET EQUAL TO PERCENTAGE OF EGGS HATCHING PLUS OR MINUS 95% CONFIDENCE INTERVAL (2X STANDARD ERROR).

X-Ray Dosage in Roentgen Units	Metaphase I Irradiated in Nitrogen	Metaphase I Irradiated in Air	Metaphase I Irradiated in Oxygen
None	$\frac{139}{148} = 93.9 \pm 4.0\%$	....	$\frac{111}{117} = 94.9 \pm 4.0\%$
396r	$\frac{306}{384} = 79.7 \pm 4.0\%$	$\frac{162}{329} = 49.2 \pm 5.4\%$	$\frac{134}{330} = 40.6 \pm 5.4\%$
1120r	$\frac{260}{475} = 55.0 \pm 4.6\%$	$\frac{31}{479} = 6.5 \pm 2.2\%$	$\frac{11}{277} = 4.0 \pm 2.4\%$
1493r	$\frac{101}{320} = 31.6 \pm 5.2\%$	$\frac{7}{309} = 2.3 \pm 1.6\%$	$\frac{0}{99} = 0$
1862r	$\frac{91}{395} = 23.0 \pm 4.2\%$	$\frac{1}{443} = 0.2 \pm 0.4\%$	$\frac{0}{470} = 0$
2450r	$\frac{21}{262} = 8.0 \pm 3.4\%$	....	....

2.4 per cent at 1,120r and 0 per cent at 1,493r. The above studies were based upon a count of 4,570 eggs.

In the prophase experiments, eggs were treated with 2,100r, 8,050r, 14,000r, 21,000r, 26,600r, 32,200r, 37,800r, and 44,100r. As in metaphase experiments, three groups were irradiated at the same dose on the same morning in nitrogen, air, and oxygen with X-ray doses up to 32,200r. Eggs X-rayed with higher doses were irradiated only in nitrogen because none of the eggs receiving 32,200r in air or oxygen had hatched.

Results of these experiments, in which 4,846 eggs X-rayed during meiotic prophase were counted, are given in table 2. The percentages of eggs hatching of those irradiated in nitrogen during meiotic prophase decreased progressively from 91.7 ±4.6 per cent of those given 2,100r to 25.9 ±5.2 per cent of those given 44,100r. When irradiated in air, the percentages of eggs hatching decreased from 95.8 ±2.8 per cent of those which received an X-ray dose of 2,100r to 8.3 ±4.4 per cent of those which received an X-ray dose of 26,600r. No eggs hatched when irradiated in air with 32,200r. When X-rayed in oxygen during meiotic prophase the percentages of eggs hatching decreased from 91.9 ±4.2 per cent when treated with 2,100r to 3.2 ±2.6 per cent when treated with 26,600r. As in air, no eggs hatched when treated in oxygen with 32,200r.

The results given above for survival of eggs X-rayed in air agree in general with those of Whiting (1945a) although exact comparisons cannot be made in every case because many of her dosages were different from those used in the present study. The percentages of eggs hatching with each type of treatment are somewhat higher than those previously published by

TABLE 2

HATCHING RATIOS OF HABROBRACON EGGS X-RAYED IN NITROGEN, AIR AND OXYGEN DURING MEIOTIC PROPHASE I. FRACTIONS REPRESENT EGGS HATCHING OVER TOTAL EGGS COUNTED AND ARE SET EQUAL TO PERCENTAGE OF EGGS HATCHING PLUS OR MINUS 95% CONFIDENCE INTERVAL (2X STANDARD ERROR).

X-Ray Dosage in Roentgen Units	Prophase I Irradiated in Nitrogen	Prophase I Irradiated in Air	Prophase I Irradiated in Oxygen
2,100r	$\frac{133}{145}$ = 91.7 ± 4.6%	$\frac{182}{190}$ = 95.8 ± 2.8%	$\frac{160}{174}$ = 91.9 ± 4.2%
8,050r	$\frac{279}{323}$ = 86.4 ± 3.8%	$\frac{137}{197}$ = 69.5 ± 7.0%	$\frac{164}{255}$ = 64.3 ± 6.0%
14,000r	$\frac{167}{225}$ = 74.2 ± 3.8%	$\frac{88}{211}$ = 41.7 ± 7.0%	$\frac{55}{204}$ = 26.3 ± 6.2%
21,000r	$\frac{173}{271}$ = 63.8 ± 5.8%	$\frac{27}{228}$ = 11.8 ± 4.2%	$\frac{18}{194}$ = 9.3 ± 4.2%
26,600r	$\frac{68}{115}$ = 59.1 ± 9.2%	$\frac{13}{157}$ = 8.3 ±4.4%	$\frac{7}{218}$ = 3.2 ± 2.6%
32,200r	$\frac{119}{310}$ = 38.4 ± 5.8%	$\frac{0}{286}$ = 0	$\frac{0}{263}$ = 0
37,800r	$\frac{130}{367}$ = 35.4 ± 5.0%	....	....
44,100r	$\frac{74}{286}$ = 25.9 ± 5.2%	....	....

the author (1954) for a similar series of experiments. The reason for this difference is explained by Dr. Anna R. Whiting's discovery that exposure of metaphase eggs to nitrogen for a long period of time can cause a decrease in the percentage of eggs hatching. Since all eggs treated in the nitrogen series of experiments previously reported were kept in nitrogen at least one-half hour, this nitrogen induced lethality was superimposed on the X-ray effects. The amount of lethality induced by nitrogen has been found by the present author to depend on more than one factor, and consistent data are most easily obtained by eliminating nitrogen induced lethality entirely. This can be done by keeping the exposure period of metaphase eggs under five minutes and it has been done in the experiments reported in table 1. Prophase eggs are either not affected by nitrogen injury or recover before they are laid.

Dose-action survival curves plotted for eggs treated during meiotic metaphase in nitrogen, air, and oxygen are shown in Figure 1 and similar survival curves for eggs irradiated in nitrogen, air, and oxygen during meiotic prophase are shown in Figure 2. The dose-action survival curves for eggs irradiated during meiotic prophase I and meiotic metaphase I in nitrogen and air are compared in Figure 3. The prophase and metaphase doses resulting in control survival in nitrogen (0r and 2000r) and 50% survival in nitrogen (1200r and 29,000r) have been superimposed in this figure. The

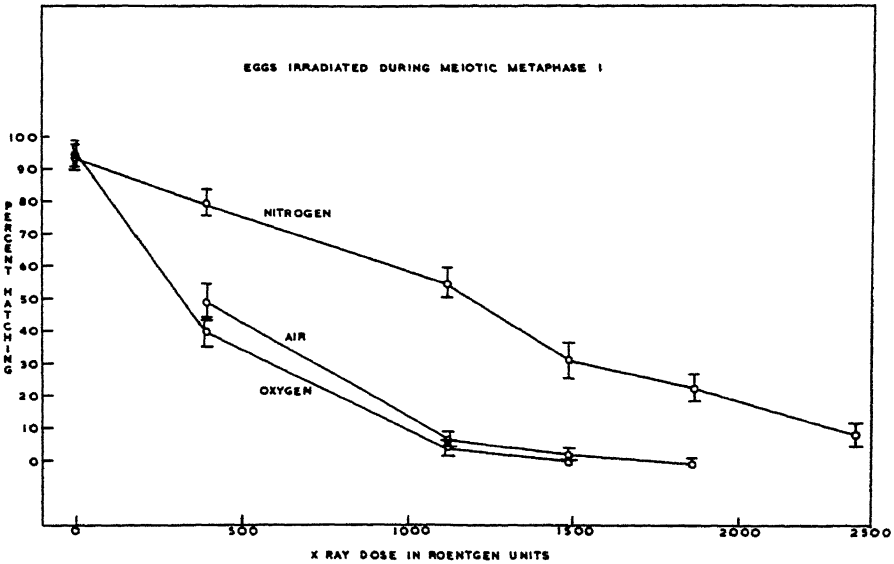

FIGURE 1. Dose-action survival curves for eggs irradiated during meiotic metaphase I. Bars represent 95% confidence intervals.

curves for irradiation in nitrogen do not differ significantly although 0% survival is reached at a lower comparable dose in prophase eggs than in metaphase eggs. This difference may reflect a difference in the type of damage causing lethality at higher doses as suggested by Whiting (1945a).

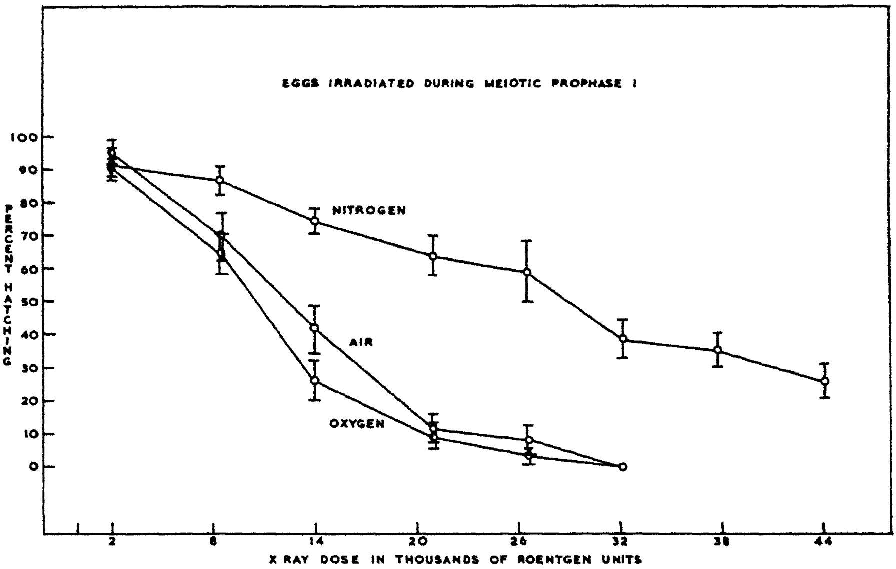

FIGURE 2. Dose-action survival curves for eggs irradiated during meiotic prophase I. Bars represent 95% confidence intervals.

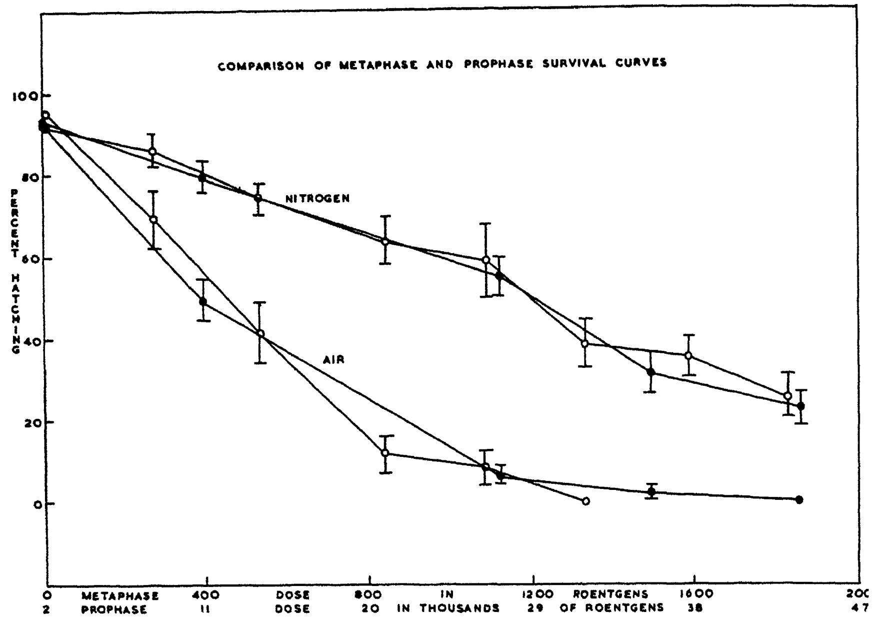

FIGURE 3. Dose-action survival curves for unfertilized eggs irradiated during meiotic prophase I and meiotic metaphase I in nitrogen and air plotted together for comparison. Solid black circles represent points for metaphase data; hollow circles represent points for prophase data. Bars represent 95% confidence intervals.

DISCUSSION

From the data presented above, it can be seen that the amount of lethality induced by a given X-ray dose is considerably lower when prophase or metaphase eggs are irradiated in nitrogen than when they are irradiated in air or oxygen. This difference increases rapidly with dose until a maximum difference is reached. In the case of metaphase eggs, the maximum difference was 48.5 per cent and in the case of prophase eggs the maximum difference was 52.0 per cent. If oxygen interferes with restitution, prophase and metaphase chromosomes must be capable of approximately the same amount of restitution. However, if this explanation is accepted, some explanation other than Whiting's tension hypothesis must be found for the extreme difference in sensitivity of the two meiotic stages. Since her tension hypothesis is well supported and fits all the data, it appears more logical to suppose that oxygen has its effect by being involved in the mechanism by which X-rays damage chromosomes rather than by interfering with restitution. The latter view is supported by cytological studies made by the author which will be published in detail elsewhere. In general terms, these cytological studies have shown that the proportion of terminal deletions and chromatin bridges present in eggs irradiated in nitrogen, air, and oxygen are the same as would be expected if these were the predominant sources of lethality. Further support has been provided by Whiting (1954) who found that all types of chromosome change (dominant lethals, recessive lethals, and visible mutations) induced by X-rays in metaphase I eggs respond similarly to the absence of oxygen at the time of irradiation.

The author therefore interprets his data to support the views of Giles and Riley (1950) and Riley, Giles, and Beatty (1951) that oxygen increases X-ray damage to chromosomes by *increasing the effectiveness* of X-ray quanta in causing initial damage. The concept that oxygen augments X-ray damage by interfering with restitution of such damage as proposed by Schwartz (1952) does not seem to fit the data obtained in the present study.

Muller (1954) feels that Schwartz's data do not provide conclusive proof of the restitution hypothesis of oxygen action. He points out that the chromosomal structures causing mosaic and recessive phenotypes in Schwartz's material are unknown and such things as two-break rings or dicentrics may be involved in the mosaics.

SUMMARY

A comparison of the effect of oxygen in modifying lethality induced by X-rays in meiotic metaphase and in meiotic prophase Habrobracon eggs has been made. Since restitution of damaged chromosomes is considered to be rare in meiotic metaphase eggs and frequent in meiotic prophase eggs, the data obtained are of interest in deciding whether oxygen has a role in initial damage to chromosomes or in preventing restitution of damage.

Studies of total embryo lethals induced in 4,570 eggs X-rayed in nitrogen, air, and oxygen during meiotic metaphase with doses ranging from 396r to 2,450r were made. The nitrogen series differed very significantly from the air and oxygen series with a maximum difference of 48.5 per cent at one dose. Similar studies were made of 4,846 prophase eggs X-rayed in nitrogen, air, and oxygen with doses ranging from 2,100r to 44,100r. Again the nitrogen series differed significantly from the air and oxygen series with the maximum differences reaching 52.0 per cent at one dose.

The oxygen effect appears to be of the same magnitude in metaphase and prophase eggs despite a considerable difference in the X-ray dose necessary to induce lethality in each of these two stages. This similarity is believed by the author to support the initial damage hypothesis of oxygen action proposed by Giles and Riley (1950) and to be contrary to the expectations of the differential restitution hypothesis proposed by Schwartz (1952).

ACKNOWLEDGMENTS

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DOMINANCE VERSUS OVER-DOMINANCE IN HETEROSIS: EVIDENCE FROM CROSSES BETWEEN OPEN-POLLINATED VARIETIES OF MAIZE*

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Arguments presented by Crow (1948 and 1952) demonstrated that if the dominance hypothesis, advanced by Jones (1917), for explanation of heterosis were correct the best hybrid that could be obtained using inbred lines extracted from an "equilibrium population" would excel that population by no more than the product of the number of effective loci and their average mutation rate. In this proposal an "equilibrium population" was referred to as one in which gene frequencies are at equilibrium between mutation and selection and genotype frequencies are those expected with random mating and independent assortment among loci. Assuming that 5000 and 10^{-5} are reasonable maxima for loci number and mean mutation rate, respectively, and that grain yield is equatable to fitness; a basis is provided for arriving at the five percent maximum superiority value for yield of a hybrid over a parent "equilibrium population." The sequence of Crow's argument, if applied to maize, then proceeds as follows:

(a) It is known that selected maize hybrids excel open-pollinated varieties in yield by much more than five percent.
(b) The dominance hypothesis fails as the explanation of heterosis.
(c) As an alternative to the dominance theory, overdominance (superiority of an heterozygous genotype over any homozygote at the locus level) is suggested as a plausible explanation.

The importance of overdominance in the explanation of gene action in maize had been advanced previously by East (1936) and Hull (1945).

The theoretical point made by Crow is relevant as a basis for inference from observations on relative performance of hybrids and open-pollinated varieties only if the latter are identical genetically, that is, in frequencies of all genes which affect yield. Differences in average grain yield between varieties and selected hybrids involving inbred lines from different varieties are not critical evidence relative to level of dominance unless the different varieties are all identical equilibrium populations. The normal procedure followed in making a double cross hybrid is to combine lines derived from different varieties. Results from Eckhardt and Bryan (1940) showed that highest yielding double crosses were obtained using single crosses of widely different parentage. If closely related lines are to be used in the same double cross hybrid, they are combined in the parental single crosses, insuring genetic divergency in the double cross.

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The observation of heterosis in a cross of open-pollinated varieties constitutes unequivocal evidence of genetic difference between the varieties. The evidence for heterosis in yield in the majority of variety crosses was conclusive in the findings in investigations prior to 1920. Richey (1922) in a review of reports on variety crosses, noted that in 244 comparisons 82.4 percent of the crosses yielded more than the average of the parent varieties and 55.7 percent excelled either parent. If variety crosses exhibited heterosis 30 or 40 years ago, as seems established in the literature, there is little reason to doubt that heterosis would be obtained in crosses of present day varieties. Nevertheless, there is a possibility that selection in the varieties in the interim has brought them to a common equilibrium status.

As a part of a series of experiments designed to study genetic differences among varieties, six southern open-pollinated varieties were compared with the fifteen possible crosses among them. The six varieties consisted of three yellow-kernel varieties—Jarvis, Indian Chief and Simpson and three whites—Weekley, Biggs and Latham.

RESULTS

The crosses and parental varieties were compared in an experiment utilizing a randomized block design with five replications at three locations in 1952 and in 1953. Data were taken on yield of unshelled maize on ten equally competitive plants in each plot. The data for yield of parents and relative performance of crosses are given in table 1.

TABLE 1

YIELD OF OPEN-POLLINATED MAIZE VARIETIES IN BUSHELS (FIRST COLUMN), IN PERCENT OF THEIR MEAN (DIAGONAL), OF THEIR HYBRIDS IN PERCENT OF THE AVERAGE YIELD OF THE TWO PARENTS (ABOVE DIAGONAL), AND IN PERCENT OF THE HIGHER-YIELDING PARENT (BELOW DIAGONAL).

Varieties		Jarvis	Indian Chief	Weekley	Simpson	Biggs	Latham
	Yield (Bu./Acre)	Percent					
Jarvis	43.4	*110.7*	129.1	119.3	116.6	106.6	109.9
Indian Chief	38.0	(121.2)	*96.9*	127.5	124.1	132.2	146.2
Weekley	44.9	(117.3)	(117.8)	*114.5*	115.4	104.6	110.0
Simpson	40.4	(112.6)	(120.5)	(109.6)	*103.1*	109.2	125.0
Biggs	38.0	(100.0)	(132.2)	(96.6)	(106.1)	*96.9*	123.0
Latham	30.6	(93.7)	(131.9)	(92.5)	(109.8)	(111.0)	*78.1*
mean	39.2						

Standard error of the percentage difference between each cross and its mid-parent. (7.3%)
Mean of crosses relative to mid-parent. (119.9%)
Mean of crosses relative to high parent. (111.5%)

The yields of the parental varieties, given in the first column of table 1, exceed the average yield of all maize grown in North Carolina during the years of this study. In general, growing conditions encountered with the various tests were satisfactory considering that rainfall was below normal in 1952 and 1953 in most areas of the state.

The italicized diagonal values give the performance of each variety relative to the average yield of all varieties. The performance of the individual variety crosses, relative to the average of the two parental varieties and to the higher of the two parents is given in the upper right and lower left portions of the table, respectively. The standard error of the mean difference between the average of two parental varieties and their cross is 7.3 percent and is applicable to the percentage difference between each cross and its mid-parent.

DISCUSSION

The results reported here are in general agreement with the early work on variety crosses; namely that heterosis does occur. In this study crosses excelled mid-parents by approximately 20 percent and the higher parent by 11.5 percent. The heterosis observed, together with the variation between varieties, is clear evidence that these varieties are genetically different and therefore the difference between mean yield of varieties and yield of selected hybrids between lines from different varieties is not critical relative to level of dominance.

Crow's argument is most directly relevant to the comparative performance of a variety and hybrid obtained using only lines derived from that variety. Unfortunately few comparisons of this type are available in the literature due to the impractical nature of this procedure of combining lines to attain maximum yield performance. Such a comparison is available in the data reported by Eckhardt and Bryan (1940) where two double cross hybrids constituted from different arrangements of four inbred lines from the Black Yellow Dent variety were evaluated with the parental variety for three years. The average yield of hybrids was approximately 14 percent in excess of the variety; which, in the light of Crow's demonstration, suggests that heterosis in maize yield cannot be explained in terms of only complete or partial dominance. However, such data are still not critical unless it is established beyond reasonable doubt that the population from which the lines were derived is actually at equilibrium (i.e. cannot be improved by selection).

The established genetic divergencies of varieties raises these questions as to how varieties differ:

(a) Are the major varietal differences due to complete absence in one variety of favorable genes present in another? If so, each might or might not represent an "equilibrium population" with respect to its own segregating loci. Such quantitative differences in gene content could result either from inbreeding (finite population size) or from divergent mutational changes in non-interbreeding populations. If the varieties are partially inbred populations, which seems entirely plausible, then what is measured as heterosis in the hybrid vs variety comparison is in part "restoration of vigor lost by inbreeding" which can (see Crow, 1948 and 1952) be accounted for on the basis of simple dominance.

(b) Are these varieties at equilibrium at all? If not, progress in yield improvement should be possible in each through intra-population selection.

The genetic variability known to exist in varieties could be of the dominance sort produced by segregation at loci exhibiting overdominance which is essentially the explanation offered by Hull (1945) for failure of selection for higher yield to be effective in varieties. However, the small (less than 0.6) estimates of the ratio of dominance to additive genetic variance led Robinson, et al. (1955) to conclude that overdominant loci are not the single important source of genetic variability in yield in the Jarvis, Weekley and Indian Chief varieties. Preliminary results from selection studies in progress with these varieties indicate that yield improvement is being affected.

A practical implication of the observed heterosis in variety crosses is that selected pairs of varieties may prove desirable foundation material for use in reciprocal recurrent selection as proposed by Comstock et al. (1949). Since two varieties can be chosen that in their original state yield a cross for which performance approaches that of commercial double cross hybrids and in which the intra-variety genetic variance is considerable (Robinson et al., 1955) the prerequisites for desirable foundation stocks appear satisfied.

The heterosis and general agronomic appearance of crosses of varieties could provide the basis for choosing the source material for deriving inbred parents of superior hybrids. Variety cross heterosis should be indicative of the average performance of all hybrids from inter-variety crosses among the inbreds of the parental varieties but may not relate to the maximum possible hybrid performance from a specific combination.

The latter would excel the performance of the variety cross itself by an amount that is dependent upon, but that probably is not predictable in any simple manner from, the genetic variation within the varieties.

SUMMARY

Observation of heterosis in variety crosses of maize makes it clear that varieties are not genetically identical. Admitting genetic divergencies between varieties, any attempt to attribute the extent to which the heterosis is accountable in terms of complete dominance *or* overdominance is largely speculation when the criterion used requires identical gene frequency for all populations. The possibility of inbreeding is suggested to account for the genetic diversity between varieties and finally it is questioned whether any intra-variety gene frequency equilibria between mutation and selection have been reached in open-pollinated maize varieties.

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A MUTANT IN DROSOPHILA PSEUDOOBSCURA WHICH ALTERS THE PIGMENTATION OF THE TESTICULAR ENVELOPE WITHOUT CHANGING THAT OF THE EYES

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ORIGIN OF THE MUTANT

The mutant to be described in the present article has the property, unique among the known mutants in various species of Drosophila, in that it alters the pigmentation of the testicular envelope without at the same time affecting the coloration of the eyes of the fly. The more usual condition is that the pigment in the testicular envelope is absent or reduced in quantity in those mutants which show also a reduction of the amount of the red pigment in the compound eyes (see, for examples, the descriptions of the various mutants in *Drosophila melanogaster* in Bridges and Brehme, 1944).

The mutant, to be called *marbled* (symbol *mbl*), was found in November, 1954 in a culture of *D. pseudoobscura* derived from a wild female collected at Bryce National Park, Utah, in the summer of 1951 by Professor Th. Dobzhansky. In the normal flies, the red coloration of the testis can easily be seen through the abdominal wall from the ventral side; in the marbled mutant the abdomen seems to be devoid of the color. Dissection of marbled males showed the condition to be due to a mosaic discoloration of the testicular envelope, which is mostly colorless except for irregular-shaped splotches of the normal red pigmentation.

In the original culture only about 4 per cent of the males showed the marbled character. By back-crossing the daughters of marbled fathers to marbled males, 54 marbled and 110 normal males, together with 213 females, were obtained. Ten of the females were then crossed individually to marbled males. In three of the cultures all the sons were marbled, showing that the females as well as the males were pure for the gene *mbl*. A pure *mbl* stock was established from these cultures.

DESCRIPTION OF MARBLED

One hundred males from the marbled stock were dissected, and all of them showed a mosaicism in the testicular envelope, consisting of bright red and colorless areas. The patterns formed by the colored areas are quite irregular and variable from fly to fly; the colored areas are, however, indistinguishable in the intensity or the shade of the color from the pigmentation of normal testes. Only a single male had one of the testes wholly uncolored; the remaining flies had some coloration in both testes. The extent of the colored areas varied from a single spot to more than half of the testicular envelope.

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One hundred females from the marbled stock were dissected, and none of them showed any colored spots on the surface of the ovaries. Among 46 wild-type females, 11 had one or more red spots on the ovaries. These spots are usually very small but easily seen under a dissection microscope; most of them are elongated in shape, but some are star-shaped or branched. In no other organ was a difference in color detected between the wild-type and the marbled flies. In particular, the two classes of flies were found to possess exactly the same coloration of the eyes. The coloration of the Malpighian tubes is also identical.

The marbled gene nevertheless does produce a pleiotropic effect. About half of the flies of either sex in marbled cultures show an abnormal position of the wings in the resting state; namely, one of the wings is held out, its axis forming a right angle with the body, while the other wing is held in a normal position. Marbled flies showing this condition are unable to fly, and when disturbed jump some centimeters. This trait is recessive; it does not appear in heterozygous females.

Reciprocal crosses between the marbled and wild-type flies showed that *mbl* is a sex-linked gene. This was confirmed by tests with the marking genes Bare, orange, and Curly, located in the second, third and fourth chromosomes respectively. No linkage with *mbl* was observed.

ABSENCE OF EFFECT ON EYE COLOR

To exclude the possibility that the gene *mbl* may have an effect on the eye color too faint to be perceived, marbled flies were crossed to flies homozygous for orange and for orange and purple. The double and triple heterozygotes were examined, and were found to have eyes of normal color. Males were then obtained which carried the gene *mbl* in their X-chromosome, and were homozygous for orange or for orange-purple. No effects of marbled on the eye colors were observed. The marbled-orange males tended, however, to have fewer and smaller colored areas in the testicular envelope than is the case in the marbled non-orange males; namely, 10 out of 30 marbled-orange males examined had wholly colorless testes. This suggests an effect of the gene orange on the testis color in the presence of marbled, while orange by itself has no such effect. Yet, this hypothesis is not tenable, since when marbled was combined with orange from another stock the proportion of males with colorless testes has dropped. It seems that genes modifying the effects of *mbl* were present in the original orange stock, but that these modifiers are not related to the orange mutant as such.

CONCLUSIONS AND SUMMARY

The discoloration of the testicular envelope in the white-eyed mutant of *Drosophila melanogaster* was one of the first thoroughly studied instances of a pleiotropic effect of a gene mutation in Drosophila (see Dobzhansky, 1927, for citation of earlier literature). Since then, mutants which affect the color of the testes have been found in several species of Drosophila. All of these mutants influence, however, also the eye color, and are, in fact, detected originally as eye mutants. More particularly, the mutants which alter the color of the testicular envelope are those with eyes of the "brown" type, which have lost or diminished the amount of the red eye pigment. It is relevant to note that mosaic discoloration of the testicular envelope is a rule in mutants which induce also a mosaicism in the eye (Plum and others in *D. melanogaster*). Although relatively few eye-color genes are known in *D. pseudoobscura*, all the previously known ones in this species obeyed the general rule. The sex-linked gene marbled described above is an exception. It influences the distribution, though not the coloration, of the pigment in the testicular envelope. It has complete penetrance, though variable expressivity. Nevertheless, it appears to have no effect whatever on the coloration of the eyes. The developmental mechanisms which bring about this condition would be interesting to study.

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LETTERS TO THE EDITORS

Correspondents alone are responsible for statements and opinions expressed. Letters are dated when received in the editorial office.

CYTOTAXONOMY OF CURCULIONIDAE (COLEOPTERA)*

The weevil genus Hylobius Germ., erected in 1817, comprises the species *congener* D. S. and M., *pales* Boh., and *radicis* Buch. in North America. In Finland and Sweden it is represented by *abietis* L., *transversovittatus* Gze., *pinastri* Gyll., and *piceus* DeG. The last named species occurs also in North America, where Leconte, considering it generically distinct, transferred it to his new genus Hypomolyx in 1876.

The North American *Hypomolyx piceus* has been found by Warren (1955) to consist of two forms, one winged and the other almost wingless, which because of the presence of other correlated morphological differences led him to entertain the idea of their being specifically distinct.† It is apparently unknown whether the vestigial-winged one occurs in Scandinavia.

Since it had earlier been established that *Hylobius congener* possesses unusually well differentiated chromosomes (Smith, 1952), the possibility of establishing the taxonomic relationship of the normal- to the vestigial-winged *piceus* on the basis of chromosome number and/or morphology seemed especially promising, and, by extension, the soundness of Leconte's introduction of the name Hypomolyx appeared open to test.

Living adult males of *pales* and *radicis*, donated by the Forest Insect Survey, Sault Ste. Marie, in co-operation with associated Forest Insect Rangers; of *abietis* and *pinastri*, kindly air-mailed from Finland by Professor Dr. Esko Suomalainen of Helsinki University; and of the North American normal- and vestigial-winged *piceus*, courtesy of Mr. G. L. Warren; and, finally, slides of a normal *piceus* male, prepared by Professor Suomalainen, make up the material briefly reported on here.

In agreement with the constitution already established for *congener*, *Hylobius pales*, *radicis*, *pinastri*, and *abietis* were each found to have 2n = 22 chromosomes: 10 pairs of autosomes and an Xy sex-determining mechanism in which the y is relatively minute and associates with the X to form a parachute ("Xy_p"; Smith, 1950). The normal-winged form of *piceus*, both from North America and Finland, is profoundly different in that it has 2n = 40 chromosomes: 19 pairs of autosomes showing little diversity in size and a typical Xy_p sex-determining mechanism. The vestigial-winged *piceus* is intermediate. It has 15 pairs of autosomes along with an X chromosome and possibly a much smaller y that, if present, is at the limit of resolution of the microscope. The autosomes, however, instead of falling into a graded series of moderate size, as they do in the normal-winged form, include two pairs that are extremely large, perhaps as much as four times the size of the remaining ones. The differences between the chromosome complements of the two forms are thus of sufficient magnitude to establish them as distinct species, one having been derived through a series of autosomal "fusions" or the other through one of autosomal fragmentation. Since "centric fusion" (White, 1954) of non-homologous chromosomes is the simpler process and since populations of another weevil have recently been encountered that comprise homozygous "fused," homozygous "unfused," and heterozygous "fused" individuals (unpub. data), the vestigial-winged form is logically regarded as being the derivative species.

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†This has recently been confirmed by Wood (unpub.).

On the other hand, the numerical uniformity of the chromosome complements of the North American and Finnish species of Hylobius (*sensu* Leconte)—there are morphological differences characterizing the chromosome sets of some, at least, of the species—and their marked difference from *piceus* clearly validate the separation of Hypomolyx from Hylobius.

The nicety of this cytological cleavage has recently been thrown open to question by a chromosome survey of curculionids published by Takenouchi (1955). This author has established the following counts: *Hylobius albosparsus* Boheman, 2n = 40; *Hylobius galloisi* Kôno, 2n = 36; *Hylobius exsculptus* Roelofs, 2n = 28; and *Hylobius montanus* Kôno, 2n = 20. Perhaps the first two will actually prove to be species of Hypomolyx, with *Hylobius galloisi* representing one of the expected "fusion" types intermediate between 2n = 40 and 2n = 32. Unfortunately Takenouchi's illustrations are in insufficient detail and at too low a magnification to allow comparative analysis, so that the significance of his findings remains at present obscure.

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THE AMERICAN SOCIETY OF NATURALISTS

Secretary's Report, 1955

The annual business meeting of the Society was held in East Lansing, Michigan, on the Campus of Michigan State University in connection with the AIBS on the evening of September 7th, 1955, with President K. V. Thimann presiding. A motion was made and unanimously passed to hold the next annual meeting with the AIBS in September, 1956, at Storrs, Connecticut.

The report of the Nominating Committee (Ernst Mayr, chairman, Bentley Glass, and Alfred E. Mirsky) was presented by the Secretary. With no further nominations from the floor the following officers were unanimously elected: President for 1956, E. Newton Harvey, Princeton University; Vice President for 1956, Frank A. Brown, Jr., Northwestern University; Secretary for 1956-1958, Bruce Wallace, Biological Laboratory, Cold Spring Harbor, N.Y.

Forty-six persons, nominated by members and approved by the Executive Committee, were elected to membership. Names of those accepting membership will be included in the roster of members to be published in the next issue of the Records. Six of the ten Honorary Members, Warder C. Allee, Charles E. Allen, Liberty Hyde Bailey, Albert F. Blakeslee, George H. Parker, and George H. Shull, died in the past year. On recommendation of the Executive Committee the following persons were elected to Honorary Membership: Benjamin Duggar, Richard Goldschmidt, J. T. Patterson, Fernandus Payne, Franklin Shull and Sewall Wright.

The reports of the Treasurer, C. P. Swanson; of the Auditing Committee, R. F. Kimball, Chairman; and of the Secretary, W. P. Spencer, were read and approved. The Society voted to approve the adoption of the Revised Constitution of the AIBS. A revision of By-law 3 of the Constitution of the Naturalists was adopted unanimously. The revised by-law reads as follows: "The Executive Committee shall be empowered to appoint an Editor for *The American Naturalist*, to serve for a five year term. The Executive Committee, in consultation with the Editor, shall appoint an Editorial Board to advise the Editor in matters of policy. Each member of the Editorial Board shall serve for a term of three years."

The Annual Report of L. C. Dunn, Editor of *The American Naturalist*, was read and approved.

A motion of thanks to the officers of the AIBS, to Michigan State University, and to Allen Fox, local representative of the Society, was passed.

At a meeting of the Executive Committee, with six of the seven members in attendance, L. C. Dunn was reappointed Editor of *The American Naturalist* for a five year term, beginning January 1st, 1956. The Executive Committee appointed the following individuals to serve as members of the Editorial Board for the three year period from January 1st, 1956, to December 31st, 1958: W. Frank Blair, M. Demerec, Libbie H. Hyman, D. D. Keck.

On invitation from the Genetics Society of America and by unanimous vote of the Executive Committee the Naturalists have undertaken to serve as cosponsor of the Tenth International Congress of Genetics, to be held in Montreal, Canada, August 20–27, 1958.

At the East Lansing Meeting on the evening of September 7th, K. V. Thimann gave his Presidential Address on the subject: "Promotion and Inhibition; Twin Themes of Physiology," a comprehensive survey of the field of plant hormones. Vice President Butler presided and introduced the speaker. On Thursday afternoon a symposium on "Modern Approaches to Problems of Differentiation," arranged by Vice President Butler, was presented. Subjects and speakers were:

Intracellular Differentiation, Tracy M. Sonneborn
A Consideration of the Determination of Tissue Cells, John P. Trinkaus
Physiological Processes Involved in the Conversion of Normal Plant Cells to Tumor Cells, Armin C. Braun
Internal Factors Controlling Cell Differentiation in Flowering Plants, William P. Jacobs

On Wednesday the Society cosponsored a symposium on "The Taxonomy of Cultivated Plants" dedicated to L. H. Bailey. On Thursday morning the Society cosponsored a symposium on "Quantification in Population Ecology." At the meeting of the AAAS, in Atlanta, Georgia, on December 28th, 1955, the Society served as cosponsor of a symposium on "The Species Problem," arranged by Ernst Mayr.

The Executive Committee of the Naturalists for the year 1956 consists of E. Newton Harvey, Chairman, Frank A. Brown, E. G. Butler, M. Demerec, W. P. Spencer, C. P. Swanson, K. V. Thimann, and Bruce Wallace. Representatives on the AAAS Council for 1956 are S. G. Stephens and one other to be appointed. The Representative on the AIBS Governing Board for 1956 is C. G. Huff.

During the past year the following deaths have occurred: E. B. Babcock, H. B. Bigelow, A. H. Hersh, Carl Moore, R. C. Osburn and F. D. Richey, in addition to those already mentioned above. The year 1955 closed with an active membership of approximately 480.

Attention is again called to the fact that on retirement from professional service an active member may become emeritus, with exemption of annual dues. Emeritus members may continue to subscribe to *The American Naturalist* for the $3.50 subscription rate paid by the Society for each active member. Request for emeritus status should be sent to the Secretary and subscription fee should be sent to the Treasurer, C. P. Swanson, Johns Hopkins University, Department of Biology.

At the end of a three year term of office perhaps a few observations are in order. It is not surprising that a biological organization, established in 1883, by a small group of American naturalists for the purpose of "discussion, advancement and diffusion of knowledge concerning the broader biological problems, including organic evolution etc.," should itself have undergone some evolution in the interim.

At the present time the Society serves three functions:

(a) as an Honorary Society in which election to membership serves as recognition of sustained research activity of a high order;
(b) as an organization for arranging and sponsoring Symposia of breadth and wide interest;
(c) as a group which controls the editorial policy of *The American Naturalist*, but does not own the journal.

Do these objectives justify the continuance of the Society? They do if the present membership, and that means each individual, will do his share toward their proper attainment. Many of you know individuals in your own or other departments who have, over a period of years, carried on a sustained research program and turned out publications of high merit, and are worthy of election to membership. If our present membership will take this matter seriously the organization can serve more effectively as a healthy stimulus to young biologists. Members wishing to make nominations should send a vita together with a bibliography, which need not be complete, but which should contain important recent publications, to the Secretary.

Furthermore there is another responsibility which comes with acceptance of membership in an organization such as the Naturalists. If you continue to achieve in your field do not resign your membership on the ground that the Society no longer has anything to offer you. Perhaps you have something to offer the Society and through it to those younger men who may benefit by the stimulus which a little recognition brings. Those of us who see the resignation lists, not large but too large, wonder sometimes why certain individuals feel that they can no longer afford to do their share toward maintaining the organization which provided for them this same stimulus, earlier in their research careers.

Perhaps you may never be asked to participate in a symposium. But you could make it a point to attend those arranged. You might even send constructive suggestions to the officers on the type of symposia which you and others would favor. It would, of course, be possible for the Society to expand its program in this field. However, examination of either the AIBS or AAAS programs indicates that there is already a plethora of symposia at these meetings. Any expansion here should look toward the setting up of regional meetings apart from these organizations.

Concerning the third objective it may be said that *The American Naturalist*, under the able Editorship of Dr. Dunn, is a journal of which we may be proud. With a strong Editorial Board representing a variety of biological disciplines, not under the domination of any one school of thought, the journal should continue to improve. As a member you may help the quality of the journal by submitting papers which contribute to biological synthesis in these days of rather narrow specialization, and by calling attention of potential contributors, who need not be members of the Society, to this medium for publication.

In conclusion your Secretary takes this opportunity to thank all those, both officers and members, who have cooperated in the work of the Society in this three year period.

Respectfully submitted,
WARREN P. SPENCER
Secretary

REPORT OF TREASURER

August 15, 1955

AMERICAN SOCIETY OF NATURALISTS

Balance, November 16, 1954	$ 939.38
Income from dues: Nov. 17, 1954, to Aug. 15, 1955	2,598.00
Total receipts	$3,537.38
Expenditures: Nov. 17, 1954, to Aug. 15, 1955	
Stamps	18.00
AIBS membership	249.50
L. C. Dunn, editorial assistance	300.00
Science Press, 496 subscriptions to *American Naturalist*	1,736.00
Realart Press, envelopes	11.00
A. F. Carr, overpayment of dues	1.50
Bank handling charges	4.51
Total expenditures	$2,320.51
Balance, First National Bank, Baltimore, Aug. 15, 1955	$1,216.87

C. P. SWANSON
Treasurer

REPORT OF THE EDITOR—1955

This report covers the period Jan. 1—Aug. 31, 1955, because of the date of the annual meeting. In this period 62 mss were considered of which 41 were published, 2 rejected, 1 withdrawn and 18 held over. The published material includes 32 articles, 7 letters to the editors, 2 book reviews and 20 pages of book notices.

The contents of the 1955 volume show a change in the direction of the desired increase in diversity of fields covered. Genetics still leads, largely because one issue was devoted to the symposium of the Genetics Society, but there were several papers on ecological, natural history and general evolutionary problems. Data papers declined in number, those with emphasis on synthesis increased, in accordance with editorial policy.

L. C. DUNN

PUBLICATIONS RECEIVED

No undertaking to publish reviews is implied in acknowledgement of publications received. Books for notice may be sent to:

EDITORIAL OFFICE, The American Naturalist
Box 2, Schermerhorn Hall, Columbia University
New York 27, N. Y.

Berrill, N. J., 1955. The origin of vertebrates. 257 p., ill. $4.00. Oxford University Press, New York.

Comar, C. L., 1955. Radioisotopes in biology and agriculture. 481 p., ill. $9.00. McGraw-Hill Book Company, Inc., New York.

Darwin, Charles, 1955. The expression of the emotions in man and animals. 372 p., ill. $6.00. Philosophical Library, New York.

Reprinted with introduction by Margaret Mead and added new illustrations of animals and men.

Demerec, M. (Editor), 1955. Advances in genetics, Vol. VII. 309 p. $7.50. Academic Press, Inc., New York.

The seventh volume of this annual publication contains the following articles: Vernon Bryson and Waclaw Szybalski, Microbial drug resistance; Adriano Buzzati-Traverso, The 'Obscura group' of the genus Drosophila; A. Brito da Cunha, Chromosomal polymorphism in the Diptera; John A. Moore, Abnormal combinations of nuclear and cytoplasmic systems in frogs and toads; Paul B. Sawin, Recent genetics of the domestic rabbit; Ryuhei Takahashi, The origin and evolution of cultivated barley.

Dice, Lee R., 1955. Man's nature and nature's man. 329 p. $5.00. University of Michigan Press, Ann Arbor.

Ekblad, Martin, 1955. Induced abortion on psychiatric grounds. 238 p. Ejnar Munksgaard, Copenhagen.

Franklin, T. Bedford, 1955. Climates in miniature. 137 p., ill. $3.75. Philosophical Library, New York.

Gray, Peter, 1954. The microtomist's formulary and guide. 794 p., ill. $10.50. McGraw-Hill Book Company, Inc., New York.

Illingworth, Frank, 1955. Highway to the north. 293 p., ill. $7.50. Philosophical Library, New York.

Imms, A. D., 1951 (reissue). Insect natural history. 317 p., ill. $5.00. McGraw-Hill Book Company, Inc., New York.

Wardlaw, C. W., 1955. Embryogenesis in plants. ix + 381 pp., ill. $7.00. Methuen & Co., London; John Wiley & Co., New York.

Professor Wardlaw discusses and illustrates embryological development in all classes of plants, and he considers to what extent visible embryonic development might be interpreted in terms of genetical, physiological, physical, and environmental factors. He is particularly interested in demonstrating how the study of plant embryology may further knowledge of plant morphogenesis, and how it may help to clarify problems of phylogeny, homologies of organization, parallel and convergent development. By way of introduction it is shown that morphogenetic principles and factors, such as genic and chemical control of development, polarity, cell division, segmentation patterns, gradients of cell size, spatial relationships, and the phenomenon of the organism as a reaction system, command attention in embryogenesis, and an experimental approach is suggested. There follow twelve chapters in which Dr. Wardlaw presents both the general morphological features and points of specific interest for Algae, Bryophyta, Psilotales and Equisetales, Lycopodineae, Eusporangiate Ferns, Leptosporangiate Ferns, Gymnosperms, and Flowering Plants. The last-named group comprises three chapters; the first deals with general features and embryonic types; the second with particular aspects, e.g., embryogenesis in primitive and advanced angiosperms, origin of the monocotyledonous embryo, parasites and saprophytes, apomixis, polyembryony; the third with analytical and experimental investigations, including embryo culture and ovular tumors. The general theme of the introductory chapters is taken up again in the final part which gives a summary of the general morphological features of embryos as well as a list of morphogenetic factors and discussion of protoplasmic organization. The bearing of embryogenesis on questions of phylogeney is also discussed here, and it is stated that "the factors which determine the embryonic development must be much more fully explored before embryological data can be used with safety in taxonomy and phylogeny."

There can be little doubt that in the past many students have been discouraged from working with the ordinarily small and rather inaccessible embryos of plants. Progress in experimental and chemical respect has therefore remained relatively small. It is Dr. Wardlaw's contention that technical difficulties may be overcome, and that general botanical problems may be considerably advanced through experimental work with plant embryos. Knowledge of the inception of embryonic patterns and of early formative processes may yet bring about better understanding of adult organization, it may also lead to a fresh outlook on the taxonomic and phylogenetic significance of morphological features.

There are numerous line drawings, text references, an extensive bibliography, and a general index.

ROBERT BLOCH

THE
AMERICAN NATURALIST

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PROMOTION AND INHIBITION: TWIN THEMES OF PHYSIOLOGY*

KENNETH V. THIMANN

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Many of us, whether zoologists or botanists, whether we are studying physiology or genetics, growth or death, working in the laboratory or in the great outdoors, are mainly measuring the rate of something and interpreting these rate measurements to advance the front of biology. Not only are we measuring rates, but we are altering them—by temperature perhaps, or pressure, or nutrition, or genetic change, or a hormone—and from the acceleration or inhibition we make deductions about our subject.

Some fortunate geneticists may claim to be exempted from this generalization, since in their cultures they may be concerned simply with growth or no growth, ability or failure to synthesize a metabolite—that is, rates infinitely low or infinitely high—yet in a surprising number of these cases it does develop that the matter is not so black and white as it seemed, and at another temperature or with some other change of conditions the missing process does go at a measurable rate.

Some morphologists may think that they are exempt from the study of biological rates, but the fact is that morphology is essentially the expression of *balance* between reaction rates. A river broadens out into lakes in flat country but narrows into a torrent on the hillside; its morphology is thus a direct expression of the *rates of flow* of water in and out. Similarly a meristem is a mass of small cells, because its cells divide faster than they enlarge; pith, or storage tissue, on the other hand, (large, thin-walled cells showing few mitotic figures) results when cells enlarge faster than they divide. We can depict this in morphological terms, that is, in photographs, or we can describe it in physiological terms, that is, in terms of *relative rates*. Such considerations, obvious in themselves, remind us that even when we think otherwise we may actually be studying the acceleration or inhibition of biological rates.

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*Address of the President, American Society of Naturalists, delivered at the annual meeting, East Lansing, Michigan, Sept. 8, 1955.

Inhibition is a universal phenomenon throughout the biological world. All sorts of substances and circumstances cause inhibitions, and all sorts of processes are subject to them. Freud was the first to show that without our personal inhibitions we would be odd and uncharacteristic creatures. By what physical mechanism these are exerted, it is as yet too soon to say. A less delicate, but still elusive example is offered by the social hormones of insects. In a hive of bees, when the queen is removed, the workers begin to produce eggs. Apparently under normal conditions egg development in worker bees is inhibited by traces of a substance produced on the outside of the abdomen of the queen, and distributed to the workers through the food (Butler, 1954). A dead queen replaced in the hive will re-establish the inhibition of egg development, at least for a time. Such dominance exercised by an excretion is doubtless limited to the insects; in small boys, dominance, with inhibition of too active competition, is exercised by physical prowess; in plants dominance of one bud over another is exercised by an internal secretion.

OPTIMUM PHENOMENA WITH A SINGLE COMPOUND

This last point brings us to a major theme, namely that inhibition and stimulation are often caused by the same substance. If a substance promotes or stimulates a process, then it commonly inhibits it in higher concentrations. The effect of dinitrophenol on the respiration of yeast is a classical example. Similarly, if a substance typically inhibits a process it commonly (not always) stimulates it at sufficiently low concentrations.

The vertebrate heart is slowed by application of acetylcholine, but if precautions are taken to lower the internal level of acetylcholine, then low applied concentrations excite it (see Welsh, 1955). Antagonists of acetylcholine may produce excitation of the Venus heart, suggesting that its normal content of the hormone is on the verge of the inhibitory level. Adrenalin shows a similar action on the salivary glands. Numerous depressant drugs are well known to cause excitation when in low concentrations. The action of auxins on plants presents some remarkably clear cases of these changes of sign.

Auxins, as is well known, promote the growth of shoots, stimulating both cell division and cell enlargement, but they inhibit the growth of roots. Inhibition is obtained at quite low concentrations (about 10^{-8} molar) with all roots tested. Yet at the lowest level of concentration, root growth is usually somewhat promoted. The effect is small, but has been observed in roots of many different plants, and particularly strongly in isolated sections of roots.

Lateral buds are inhibited by the very same auxin concentration which stimulates the growth of the axis on which these buds lie. Thus they remain inhibited so long as the terminal bud goes on producing auxin. As the terminal bud grows away from them the auxin supply becomes weakened by distance, so that eventually laterals far down the stem begin to grow out.

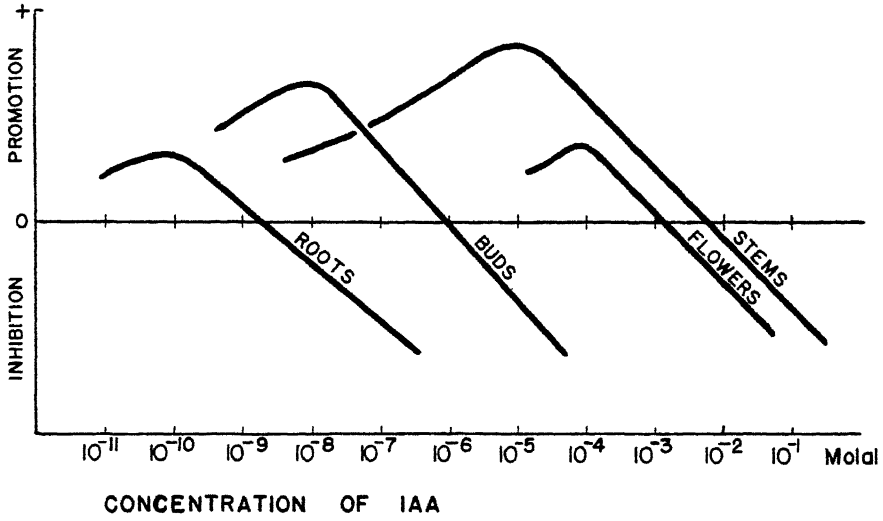


FIGURE 1. The effect of indole-acetic acid (IAA) on the growth of different organs (diagrammatic). From Leopold and Thimann, 1949.

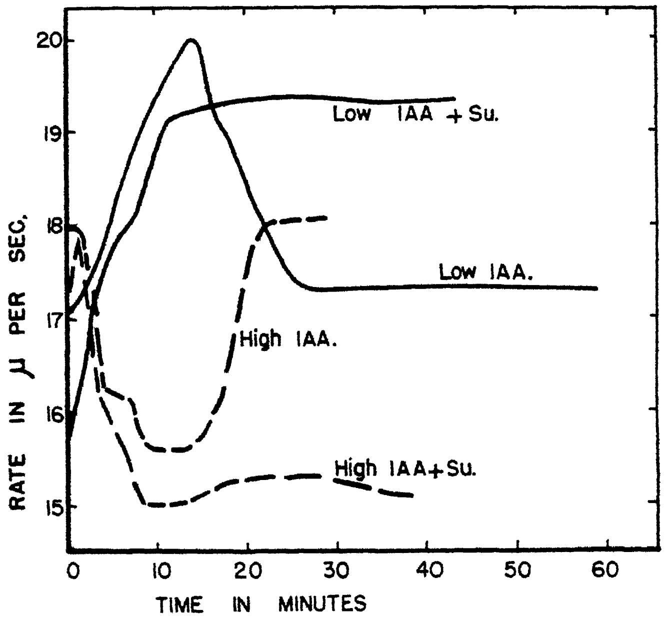


FIGURE 2. Changes in the rate of protoplasmic streaming in epidermal cells of the Avena coleoptile. Auxin (IAA) is added at time zero in each case, and the rates of streaming determined every 1–3 minutes thereafter. Note that IAA alone causes an increase or decrease in rate which soon returns to normal, while IAA and sucrose (Su) causes an increase or decrease which is maintained. Redrawn from Sweeney and Thimann, 1938.

This is an example of *weakening of inhibition with increasing age*, but it obviously has a simple explanation, namely that access of the inhibitor falls off. (True change of inhibition with aging does occur also, however, and will be mentioned below.) The relations between growth and auxin concentration for different organs are expressed in figure 1.

A nice example of promotion and inhibition in the same cell is given by the effect of auxin on protoplasmic streaming in epidermal cells of Avena coleoptiles. At low concentrations, streaming is markedly accelerated, but at about 5.10^{-5} molar and above the rate is decreased, and in the presence of sugar it may remain depressed for hours (figure 2).

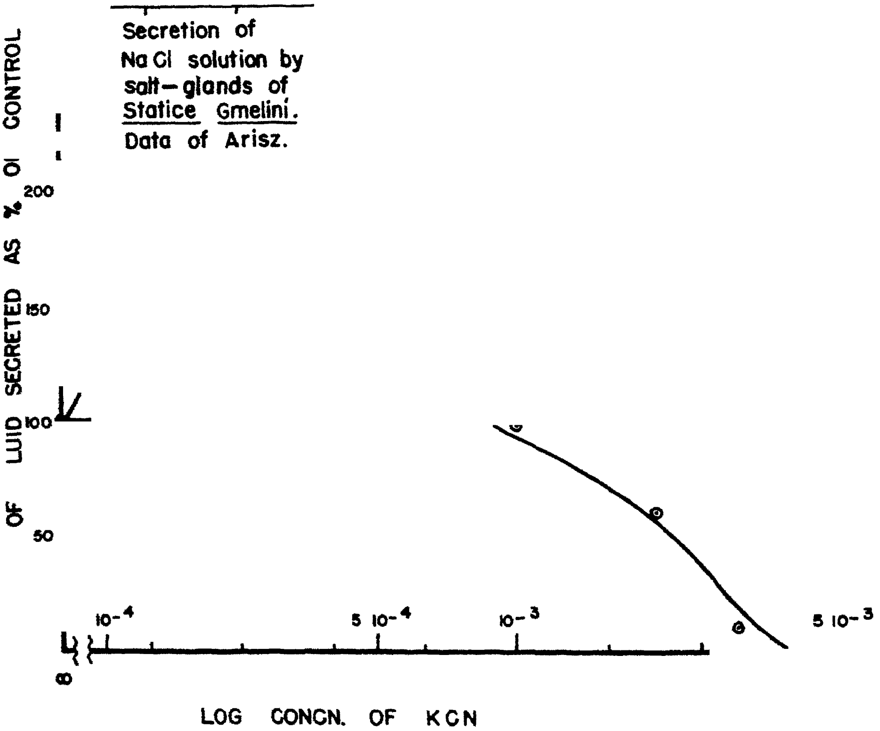


FIGURE 3. Inhibition and promotion of salt secretion by salt glands of Statice, caused by potassium cyanide. Plotted from data of Arisz, Camphius, Heikens and van Tooren (1955).

Where such clear optima are concerned it is hard to say whether we are dealing primarily with a stimulating substance which inhibits at high concentrations, or with an inhibiting substance which stimulates at low concentrations. One thinks of auxin as a stimulating substance because growth stimulation is its most typical quality. But consider typical inhibitors of enzymes. Cyanide inhibits cytochrome oxidase, and as a result it inhibits respiration and processes dependent on respiration, but in low concentrations it promotes some of these. A recent striking example is its large promotion of salt excretion in the glands of Statice (Limonium) plants, studied by Arisz et al. (1955). Concentrations of cyanide about five times the promoting level inhibit in the normal way (figure 3), and these are in the range where respiratory inhibition would be expected. Oxygen itself shows a marked optimum curve for growth of shoots and coleoptiles, air being clearly too high, and the maximum growth rate occurs at 3–8 percent O_2 (Ranson and Parija, 1955). This reaction is apparently due to effects on the auxin system.

Optimum phenomena used to be referred to in the German literature as the Arndt-Schulz law, having been formulated by Arndt in 1882 from experiments on animals, and by Schulz in 1888 from studies on yeast fermentation. The "law" can be paraphrased: Every poison causes either a reduction or an increase in physiological performance, corresponding to the larger or smaller "intensity" of its stimulus, that is, its concentration. Of course, there are many exceptions. Many enzyme inhibitors show no promotion at all, particularly when acting on isolated enzyme systems. On the other hand in one case a *double* change of sign has been reported; aspartic acid in very low concentration promotes growth of certain tissue cultures; higher concentrations inhibit, but very high concentrations promote again (Riker and Gutsche, 1948).

In a recent discussion of the numerous growth inhibitors for bacteria (Thimann, 1955), these substances were divided into six types: (1) substances forming complexes with the heavy metals of enzymes, for example, HCN, CO, 8-hydroxy-quinoline, α-α-dipyridyl; (2) sulfhydryl reagents; iodoacetate, heavy metals, especially in organic form, arsenite, mustard gases, unsaturated lactones, and probably the oxidizing radicals formed by X-rays and other radiation; (3) soaps, phenols, detergents and similar compounds which act by being adsorbed at surfaces and membranes; (4) dyes, especially active on Gram-positive bacteria and fungi; (5) substances structurally resembling essential metabolites—an enormous group, ranging from malonic acid through sulfanilamide to the antivitamins and recently antiauxins; with the general characteristic that the inhibition they cause can usually be relieved by excess of the normal metabolite and hence is labeled "competitive"; (6) the inhibitors from natural sources, like the antibiotics, whose mode of action is generally unknown.

Group 2, the sulfhydryl reagents, offer some very marked reversals of effect. For example, as far back as 1927 Niethammer showed that silver, lead, and other metals promote the growth of Aspergillus very considerably at low concentrations, while they inhibit at high. Inhibition usually sets in at $\frac{M}{1000}$ to $\frac{M}{10{,}000}$, depending on the metal used; promotion is optimal at about 1/10 this level.

The effect of iodoacetate on growth of Avena coleoptile sections is also striking; low concentrations can increase growth by as much as 60 percent (figure 4). Note, however, that promotion depends on the age of the cole-

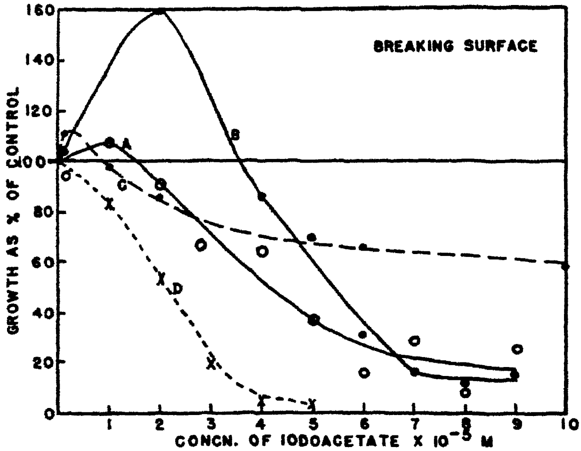


FIGURE 4. Inhibition and promotion of growth of isolated sections cut from Avena coleoptiles of different ages, caused by iodoacetate. Curve A 74 hrs., B 64–66 hrs., C 54 hrs., D 96 hrs. From Thimann and Bonner, 1948.

optile. Very old coleoptiles show only inhibition, while young coleoptiles are generally insensitive and show little promotion or inhibition. Organic mercurials show similar effects but the extent of growth promotion is smaller. Growth promotions have been recorded as a result of X-radiation too.

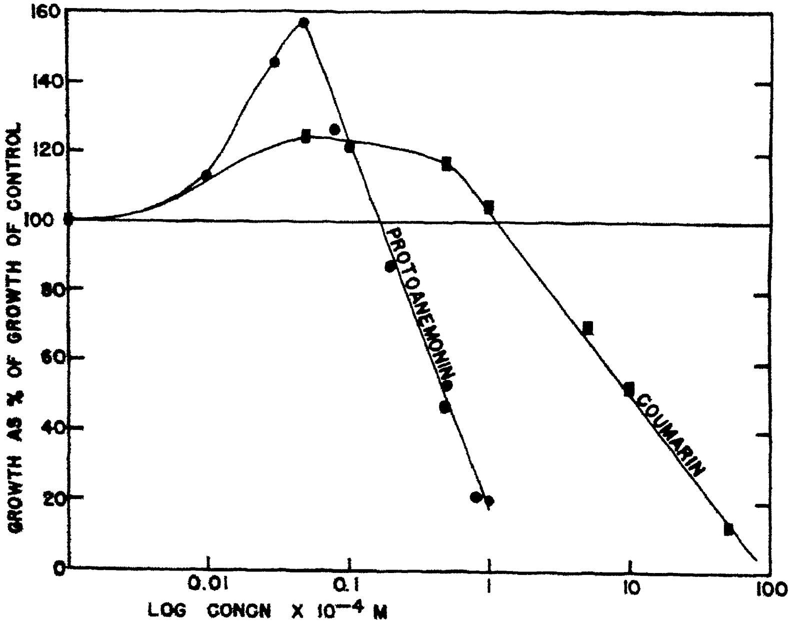
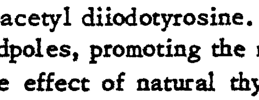


FIGURE 5. Inhibition and promotion of growth of coleoptile sections caused by two unsaturated lactones. From Thimann and Bonner, 1949a.

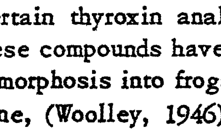
These effects make one wonder whether perhaps plants contain a natural growth inhibiting system, which is excessively sensitive to SH reagents. If such a system exists, then normal growth would be due to a balance between it and the growth-promoting hormone system.

Still more striking are the effects of unsaturated lactones such as coumarin. These compounds also react with SH groups, as is shown by the ease with which the growth inhibition they cause can be reversed by BAL (2,3-dimercaptopropanol-1). Their action on the coleoptile is characterized by massive growth promotion at a wide range of low concentrations (figure 5). In these cases, one is at a loss to decide whether these compounds are really to be regarded as growth promoters or growth inhibitors.

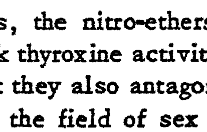
I suspect that at least some of these unsaturated lactones represent a special case. From the study of the auxin activity of many compounds it has become clear that the structural requirements which a compound must possess to have activity include an unsaturated ring, a sidechain bearing an acid group, and a certain spatial relation between these two. (Certain other planar structures may take the place of a ring.) In acids with an unsaturated sidechain, this spatial relation is shown in the fact that the *cis* isomer is active while the *trans* isomer is not. *Trans*-cinnamic acid and some of its derivatives, *trans*-naphthaleneacrylic acid and *trans*-tetralidene-1-acetic acid are inactive—their *cis* enantiomorphs all show varying degrees of activity (see Veldstra, 1953). Now a lactone is of necessity derived from a *cis* acid (by condensation with a hydroxy-group) so it is an unsaturated ring compound of *cis* configuration. It is, therefore, suggested that the *growth promoting* part of the action curve of these substances is due to their close similarity to the auxin structure. Thus these, and perhaps some other compounds too, are to be considered as simultaneously *both* promoters and inhibitors.



Trans-(inactive)

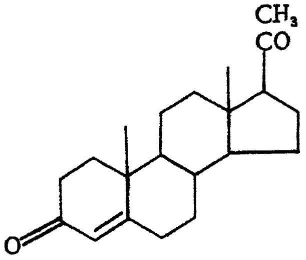


Cis-(active)

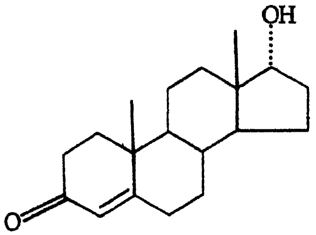


Lactone

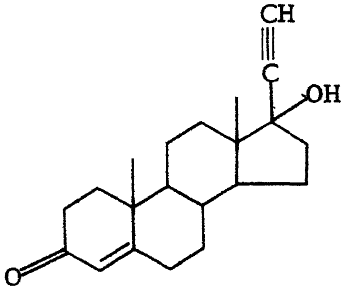
Such a combination of opposing properties is not unknown in other fields. An example is given by certain thyroxin analogues, the nitro-ethers of N-acetyl diiodotyrosine. These compounds have weak thyroxine activity in tadpoles, promoting the metamorphosis into frogs, but they also antagonize the effect of natural thyroxine, (Woolley, 1946). In the field of sex hormones the strange case of pregneninolone must be remembered; this compound possesses progestational, estrogenic and androgenic activities, in spite of the fact that the first two are clearly opposed to one another in their action on the uterus, while the second and third are certainly opposed in their general activity (see Courrier, 1950). Testosterone also has the same three activities. Here the structural requirements for activity differ so little between the three hormonal types that one can well imagine the same compound simultaneously satisfying the requirements for all three.



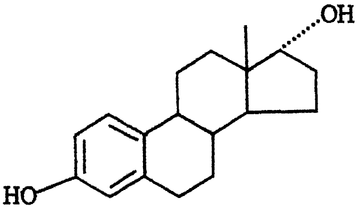
Progesterone
(Gestagenic)



Testosterone
(Androgenic)



Pregneninol-17-one-3



Estradiol
(Estrogenic)

INTERCHANGE OF PROMOTION AND INHIBITION: BIOLOGICAL BALANCE

Another characteristic of inhibition and promotion is that the relation between the two is easily altered. We have seen examples of its dependence on age. It is also changed by *duration of experiment.* The most familiar example of this is the development of resistance. Bacteria treated with an antibiotic mutate to a resistant or independent form, which is able to grow rapidly in the virtually uninhabited medium. The result is that very soon the inhibition has disappeared and the organisms (carrying a single mutation but otherwise recognizable as the original organisms) appear as flourishing as ever. A particularly nice example is furnished by the housefly treated with DDT. This animal develops resistance by the simple expedient of producing a dehydrochlorinase which splits HCl out of the DDT and thereby renders it harmless. Fortunately, not many insects are able to do this, or our control problems would be seriously multiplied.

But inhibitions of growth change into promotions sometimes without the intervention of mutant forms. Aspergillus, inhibited in growth by a fungicide, 2,2′-methylene-bis(4-chlorophenol), shows recovery after some days. By the time the cultures are 15 days old the fungicide is clearly promoting growth in the lower concentrations. Such recovery only occurs when the nutrient is supplied at high concentration (table 1). This promotion could, of course, be due to a greater inhibition of respiration than of growth, so that after a while more of the substrate becomes available for growth in presence of the fungicide than in the control. This would only be likely to occur when the substrate level was high enough so that it would not have been exhausted in the early days of the growth.

There are, indeed, numerous cases where inhibition or promotion results according to the substrate or energy source supplied. Niethammer (1927) found that manganese, lead and an organic mercury compound only stimulated the growth of *Aspergillus niger* when ample sugar was supplied. When the sugar was more nearly in limiting concentration these metals only inhibited growth, at all concentrations (*cf.* the fungicide above). A curious example arose recently in a study of the action of cobalt on the growth of plant sections. Miller (1954) had reported that cobalt alone promotes elongation of pea stem segments only weakly but that if sugar is added the promotion is much enhanced. It now develops, especially with Avena coleoptiles, that the cobalt effect has a quite different character with different substrates (Thimann, 1956). With sugar there is a clear optimum, along with inhibition at high cobalt concentrations (probably due to interaction with a sulfhydryl enzyme). With malate no such optimum is found, but the highest cobalt levels continue to increase the growth, though the whole effect is much smaller than with sugar. With acetate plus sugar, on the other hand, there is practically no growth promotion, and almost all cobalt concentrations inhibit strongly (Figure 6). This behavior may best be inter-

TABLE 1.

INHIBITION AND GROWTH PROMOTION OF *ASPERGILLUS NIGER* BY A BIS-CHLOROPHENOL. (DATA OF WATKINS AND CLEMME, 1948).

	Mycelial weight in 15 days		
Concentration of Dextrose:	1%	4%	8%
Control	210	390	390
With fungicide, 30 p.p.m.	110	380	535
As percent of control	52	97	137

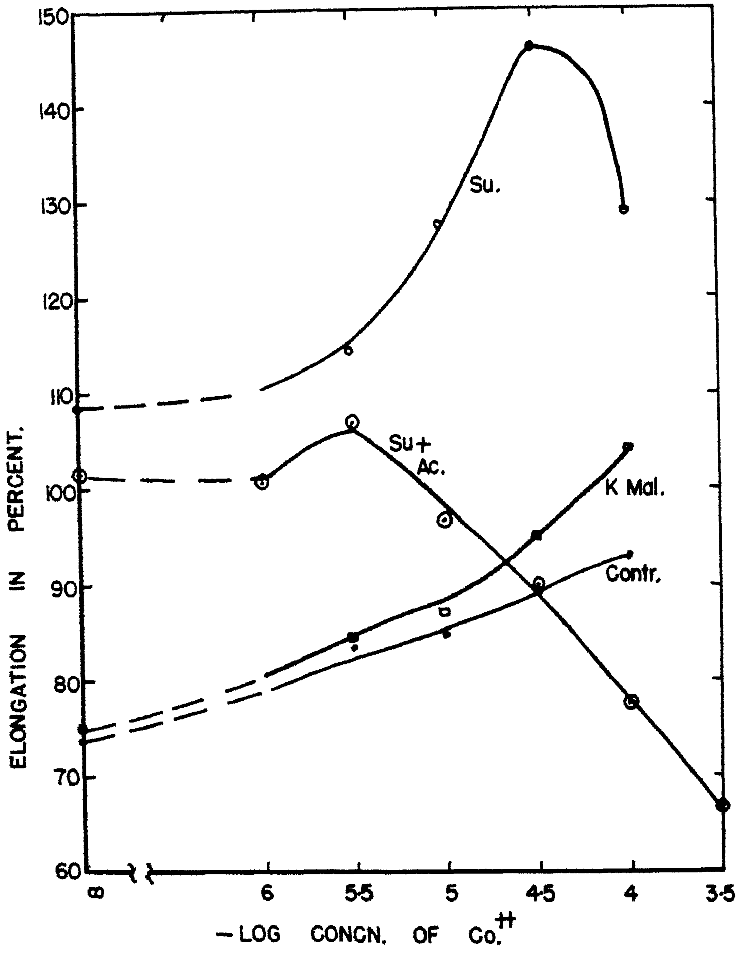


FIGURE 6. Inhibition and promotion of growth of coleoptile sections by Cobalt as function of the carbon source. Su = sucrose 2%, (0.06M); Ac = potassium acetate 2.10^{-3}M plus Su 2%; KMal = potassium malate 10^{-3}M; Contr = control. All solutions contained auxin (IAA) at a concn. of 3 mg. per liter. Redrawn from Thimann, 1956.

preted in terms of competition between different metabolic paths which are affected in different ways by stimulation of the same enzyme.

In the intact cell (or tissue) there are likely to be a number of metabolic systems influencing any one process, some promoting, some inhibiting or delaying. The action of any given inhibitor or promoter, even if it seems as simple as that of cyanide, must therefore be viewed in terms of altering a balance between opposing forces.

We can get an idea of how the cell achieves such balances from the well worked-out effects of dinitrophenol (DNP) and its congeners. Classically, these compounds stimulate respiration in Arbacia eggs, yeast and animal tissues, but inhibit it at high concentrations. The explanation now generally accepted is that DNP acts by inhibiting the phosphorylation that is normally linked to respiration. The oxidation of every molecule of hexose leads to the formation of 30 to 40 molecules of ATP by the phosphorylation of the diphosphate, ADP. As a result there is normally an overproduction of ATP by respiration, and a permanent shortage of ADP. However, a few critical reactions are dependent on the presence of ADP as acceptor of phosphate residues; the respiration rate is therefore limited by ADP (or other phosphate acceptors). Hence by inhibiting phosphorylation, nitrophenols make ADP available and respiration can increase. At really high DNP concentrations, so much ADP becomes present that even fermentation can start. Now a second acceleration is superimposed, for fermentation (when it does begin) uses up sugar even faster than does respiration (figure 7). Thus DNP causes a tremendous increase in sugar consumption. But the prevention of phosphorylation means that, at all concentrations, synthetic or assimilatory processes (*assim.* in fig. 7) are inhibited. Only at very high concentrations are fermentation and sugar usage inhibited, presumably because of a too powerful interference with phosphorylations.

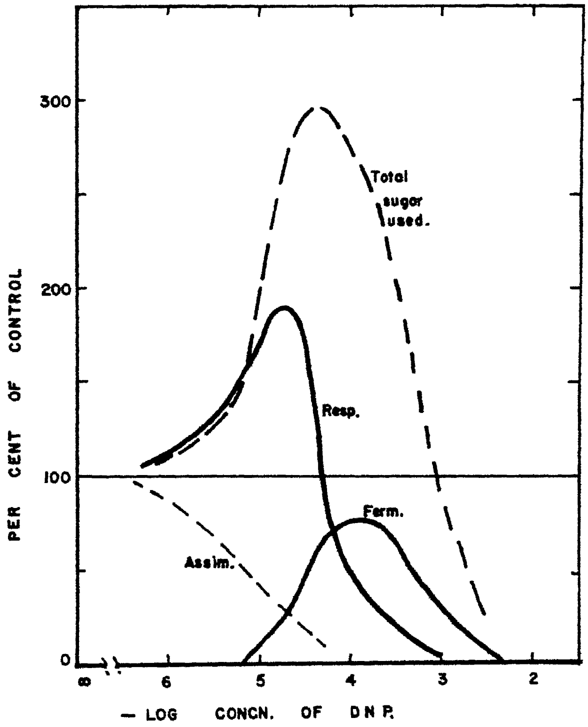


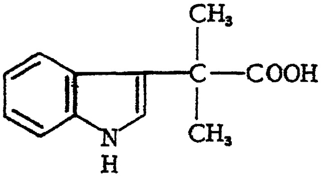
FIGURE 7. The effects of 2,4-dinitrophenol (DNP) on sugar metabolism by a baker's yeast. The rates of fermentation are expressed as percentage of the CO_2 production of the control. Replotted from figures of Simon (1953).

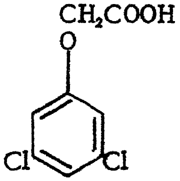
The action of thyroxin has of course been compared to that of nitrophenol; it increases respiratory rate apparently by making phosphate acceptors available, as both Martius and Lipmann have shown. It thus decreases syntheses of many kinds, including that of fat. Yet low, normal thyroxin concentrations are essential for normal activity, and they actually stimulate those syntheses which are dependent on ATP. The effect of thyroxin on syntheses must, therefore, be represented by an optimum curve. To explain this, Hoch and Lipmann have suggested (1953) that if the linkage between respiration and phosphorylation is very tight then all the hydrogen of organic compounds must be oxidized to H_2O, yielding ATP, whereas in order to achieve normal syntheses some hydrogen must be available for chemical reductions. In other words, too tight a linkage is no more desirable than too loose a one, but both hydrogen and ATP (may we say both matter and energy?) must be available. Put in another way, a small decrease in the energy yield, by making hydrogen available for reductions, actually causes an increase in the syntheses.

LIMITING FACTORS

The promotions of respiration discussed above raise some tantalizing questions about limiting factors. Most plant and animal tissues, if treated with glucose, increase their rate of respiration. We can therefore conclude that respiratory rate is being limited by the glucose, or shall we say in general by the SUBSTRATE. But now respiration is very sensitive to cyanide, or to CO in the dark. These agents inhibit cytochrome oxidase, the terminal oxygen-activating enzyme. From this we deduce (contrariwise) that the factor limiting respiration must be the OXIDASE. Worse, however, we have just seen that nitrophenol increases respiration by making phosphate acceptors available, from which we deduced that respiration is limited by the PHOSPHATE ACCEPTORS, and that the substrate must be in excess. In the case of yeast all three experiments have actually been done on the same organism. It certainly looks as though there must be something wrong with our reasoning. As far as cyanide is concerned we can perhaps extricate ourselves from the tangle by making the assumption that the oxidase was not really the limiting factor, but that doses of cyanide too low to show any inhibition already combine with the oxidase, and only as the cyanide dosage is increased is the point finally reached at which the amount of functioning oxidase has been cut down to where it does become the limiting factor. This could of course be tested. But as for the other two, limitation by substrate and by phosphate acceptor, they seem virtually incompatible. One is reminded of the amorous poet's lament, "how happy could I be with either, Were t'other fair charmer away."

Inhibitions of another sort, but based also on competition, have recently come into prominence in plant physiology, for some of the substances which structurally resemble auxins act antagonistically to auxin, inhibiting growth in shoots and increasing it in roots. Among the most effective compounds for increasing root growth are indoleisobutyric acid and 3,5 D:





It is not my purpose to review this extensive field here; it is much too long and too technical, but one or two comments may be worth making. One is that in this, or any other hormone-antihormone system, it is essential to keep in mind the concept of limiting factors already mentioned. Kinetic analyses have been used to show that there is the same strict competition between auxin and antiauxin in the growth of test plants as, for example, between succinate and malonate in the extracted succinic dehydrogenase enzyme. The demonstration essentially depends on the fact that the higher the concentration of auxin the less marked is the inhibiting action of the antiauxin. But now consider what are the limiting factors in growth. At low auxin concentrations, it is presumable that auxin is the limiting factor, and anything which interferes with its action would have maximal effect. At high auxin concentrations the growth rate is more nearly limited by the energy supply, or the rate of entry of water through the cell membranes, etc., so that an auxin antagonist would have minimal effect. Thus the reduced effectiveness of the antagonist in presence of high suxin need not be due to its actually being *displaced* by auxin, but only to its acting in a system where auxin is not the limiting factor. In this way, the competitive nature of the inhibition may be only apparent. The complementary case is the one in which an inhibitor acts only on the energy source, and therefore should have a much greater effect at high auxin levels (where the energy source would be limiting) than at low ones. Exactly this behavior has in fact been demonstrated for growth inhibition by arsenite (Thimann and Bonner, 1949b).

In roots the relationships are very complex. Auxin alone inhibits the elongation. Antiauxins promote root elongation, presumably by antagonizing the auxin. But a second antiauxin added at the same time appears to antagonize the first, decreasing the growth again (Hansen, 1954). It seems almost impossible to explain this on the ground that auxin and the antiauxins function at the same site. Burström has suggested that perhaps the antiauxins act also on the system causing auxin uptake. If they inhibited auxin uptake, of course, they would stimulate growth in added auxin, and if they inhibited each other's uptake they would antagonize each other. Perhaps it is suggestive that triiodobenzoic acid—an antiauxin by many standards—has recently been shown to inhibit the transport of auxin (Kuse, 1953; Hay, 1955). Transport from cell to cell certainly has much in common with uptake which is transport from solution to cell. Furthermore Reinhold (1954) has shown in stem tissue that one auxin (2,4-D) does inhibit the uptake of another (indoleacetic acid). One is tempted to speculate that biological antagonisms, at any rate those between externally applied hormones, may in many cases be exerted on the absorption rather on the hormone-controlled process itself. This will certainly bear examining in the near future.

BASIS OF THE OPTIMUM CURVE

It only remains to propose an explanation for the optimum curve in at least the special case of auxin. One explanation has already been given, based on the idea that the auxin molecule causes growth by combining with two other molecules, namely an enzyme and a substrate, which it thus brings together (Skoog *et al.*, 1942). If the auxin is in excess, the enzyme molecules combine with some of them while the substrate molecules combine with others. Thus the enzyme and substrate are not brought together but held apart. This explanation is certainly possible, but the following one is believed to be more general in nature.

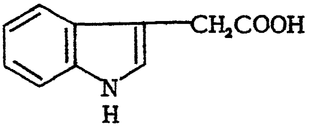
Let us suppose that the auxin does not cause growth by combining directly with its receptor molecule or enzyme, but only after conversion to a modified form. (Such a form might be indoleacetyl phosphate or indoleacetyl co-enzyme A, to mention only two possibilities.) If the auxin is in excess, much of it cannot be converted to this modified form because the *converting system* (phosphokinase or other enzyme) is saturated. Hence the unconverted, inactive auxin is in competition with the active converted form, and may thus inhibit the growth reaction, just as glyceraldehyde inhibits the fermentation of phosphoglyceraldehyde. In this way the different optimum curves for different plant organs (Figure 1) could be explained by different levels of a single enzyme, namely the enzyme of the *converting system*. This theory may prove a very useful one, since it is capable of wide application, and could explain the optimum curves given by a great variety of vitamins, hormones and drugs.

RELIEF OF INHIBITION

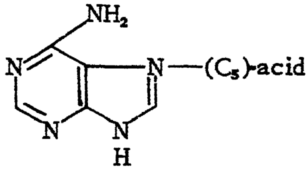
Where inhibition is due to combination with a sulfhydryl enzyme or coenzyme it can sometimes be prevented or relieved by protecting the SH group. This was shown long ago by Hopkins when he protected succinic dehydrogenase against maleic acid by adding glutathione. In growth experiments, plant sections could be protected against the coumarin inhibition by administering the sulfur compound BAL, as mentioned above. If in a similar way we could protect against the inhibition by high auxin concentrations this would give a definite lead to the site of action of auxin, a key botanical problem and one which indeed remains unsolved for nearly all hormones. Two interesting advances have been made in this direction recently.

Growth of the fungus *Nectria galligena* is inhibited by high auxin concentrations (20 to 100 mg. of indoleacetic acid per liter) and this inhibition is prevented or relieved by an extract of cotton or better still by biotin (Nysterakis, 1954). As little as $1/10\gamma$ biotin counteracts 100 mg. auxin.

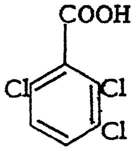
The inhibition of bud development by auxin has been especially studied in growing tissue cultures where auxin inhibits bud *formation*, a similar phenomenon but one more open to experiment. Here the inhibition can be largely relieved by adenine (Skoog and Tsui, 1951, Miller and Skoog, 1953). Phosphate increases the action of adenine and adenosine is also effective. In presence of adenine, other purines and pyrimidines increase the effect. 6-Furfuryl adenine is much more effective than adenine. From this and other work it has been concluded that the inhibiting action of auxin is exerted on nucleic acid formation. Much confirmatory evidence points in the same direction. The morphology of tissue cultures producing buds and roots depends in large part on the auxin-adenine balance and there are now several recorded interactions between auxin and adenine or other purines. After considering structural relations between auxins and antiauxins it is tempting to speculate further on the purines. No one seems to have noticed the very suggestive similarity of the formulae:



Indoleacetic Acid (IAA)

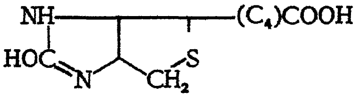


Adenosine



2,3,6-Trichlorobenzoic acid

Is it too far-fetched to suggest that such compounds might react at the same enzyme system? The formulae are certainly not further apart than IAA and some synthetic auxins, for example, trichlorobenzoic acid, which is as active as IAA. And could one perhaps, even more fancifully, implicate biotin:



Biotin

I believe these possibilities should be taken seriously.

SYNERGISM

One curious phenomenon which often goes along with inhibition is synergism. Some auxin-like compounds increase the effectiveness of low concentrations of auxin. Triiodobenzoic acid is perhaps the best example, causing synergism in inhibiting the growth of roots and in promoting growth of coleoptiles and especially curvature of pea stems. Synergists frequently have all but one of the structural features of the molecule necessary for auxin activity, as was pointed out by Went (1949). Similar phenomena are known with the steroid hormones, particularly the action of progesterone in increasing the response of the uterus to estrogens (see Courrier 1950). How are they to be explained? For if the hormone-like substance combines with the same locus as the hormone it can only antagonize. In the special case of auxins it is interesting to note one point: synergism is larger and easier to obtain in the pea test than in growth of Avena coleoptiles. And it has long been known that the pea test is less specific for synthetic auxins than tests using Avena coleoptiles; a wider range of substances shows activity in peas, and the activity of a given synthetic compound is almost always relatively higher in peas. With this preliminary a suggestion can be made about auxin synergism. For if the requirements for activity are less stringent in one plant than in another, there must be a difference between the combining sites in the two plants. For instance, the combining sites in the pea plant might be more flexible. But there is another and more interesting possibility: the combining sites might be more *varied*. There seems no necessity to assume that all combining sites in one plant are the same. Suppose there are a family of them, closely similar but not identical. Some fit the auxin molecule exactly, and combine with auxin rapidly and with high affinity. Some fit less well and hence combine more slowly and/or with lower affinity (Figure 8). But they fit better with another substance similar to, though not identical with, auxin. Hence this other substance,

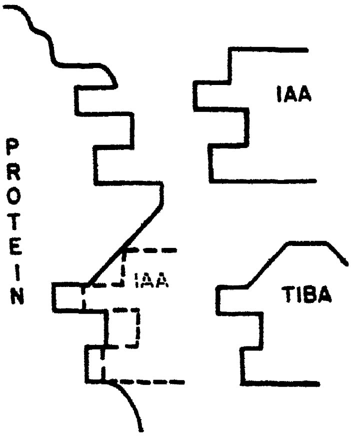


FIGURE 8. Synergism. The enzyme (left) is depicted as containing combining sites of slightly different morphology. The auxin (IAA) and synergist (TIBA) combine preferentially each at a different site.

for example, triiodobenzoic acid, will combine preferentially with these less closefitting sites and as a result it will displace the auxin from them. Auxin, on the other hand, will displace the triiodobenzoic acid from the sites where the auxin molecule fits best. As a result, in presence of the synergist more auxin molecules will become combined at their most effective sites; the auxin molecules will hence function more effectively and more growth will ensue. Of course if the synergist concentration is too high it will begin to interfere with the combination of auxin at its effective sites. Thus the synergists all inhibit growth at high concentrations.

Considerations of limiting factors are of interest here too. It is evident that the action I have described would only be effective when the auxin is in low, and limiting, concentration. At high auxin concentrations all the effective centers are occupied and the synergist can do no more. A nice example is seen in the rooting of certain cuttings, in which indole, which is quite inactive in itself, synergizes with indoleacetic acid. At low indoleacetic concentrations, 2 to 18 ppm., the indole about doubles the number of roots; at high concentrations (50 to 100 ppm.) it scarcely increases them at all (van Raalte, 1951). Yet this is certainly not to be attributed to competition; indeed almost the reverse. The root-forming ability is simply limited, at high auxin concentrations, by other factors. So the evidence which is often taken as showing strictly "competitive" action may not really show this, but may be a natural consequence of the interaction of a group of limiting factors in controlling a multifactor process.

CONCLUSION

Thoughts and speculations of this kind are, one hopes, occasionally justified if they assist in the visualizing of a number of apparently unrelated phenomena as a *group*, with a common pattern, or if they help one to discern a biological principle in a pile of experimental data.

The principle that inhibition is often or usually accompanied by a change in the balance between opposing reactions seems to emerge with clarity: promotion of one reaction can be due to inhibition of another and vice versa, through the resulting removal of limiting factors, or the accumulation of reagents which previously were rate-limiting. Overall processes like cell enlargement or the synthesis of complex compounds appear to be commonly subject to multiple control. It is this, probably, which gives the cell its enormous flexibility, and helps the organism to survive under a variety of unfavorable conditions. It is this which lies at the heart of the cancer problem, where a small and subtle change in the balance between growth promotion and inhibition leads to a long-continued overgrowth. Perhaps indeed it is this interaction and balance between multiple systems that is the real distinguishing characteristic of living organisms.

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INTERNAL FACTORS CONTROLLING CELL DIFFERENTIATION IN THE FLOWERING PLANTS*

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The idea that one chemical, or perhaps one physical process, normally controls the differentiation of a given cell type is common coin in biology. But though the idea is common, substantial evidence in support of it is rather rare. This research was started in the hope of finding extensive and quantitative evidence linking a naturally occurring substance to the differentiation of a given plant cell type.

To make the job easier, the substance was picked first and then a search was made for a cell-type to go with it. Auxin, the plant growth hormone, has two useful characteristics: (1) it has been reported by many researchers to show strictly polar movement and (2) amounts of auxin can be measured in an excellent, quantitative bioassay called the "Avena test" (cf. Went and Thimann, 1937). Since the reported strictly polar movement of auxin (from the original tip to the original base, irrespective of gravity) is so unusual, I scoured the botanical literature for reports of some cell-type which showed a matching polar differentiation. Such a cell-type was found in xylem, the cells which serve as channels for the transport of water and salts. Xylem cells occur in strands which run up and down the stem and out into the leaves. If one of these strands of xylem cells is cut, a number of investigators have reported that the xylem strand regenerates, but only toward the bottom of the stem. The new xylem cells differentiate by quite direct transformation of the unspecialized cells called parenchyma. In this transformation, a new cell wall is laid down inside the old one, and often portions of the old wall are dissolved to form holes directly connecting the lumen of one new xylem cell with that of the next.

QUALITATIVE RESULTS

By cutting off leaves and pieces of stem, using agar blocks to collect auxin from their cut surfaces, it was found that little or no auxin was produced by the stem, but large amounts were produced by the leaves—

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particularly the leaves which were growing the fastest (fig. 1). This was expected from the results of earlier workers on other plants.

But when we made a routine check of the auxin transport in Coleus stems, we found to our discouragement that auxin movement was not strictly polar: significant amounts of auxin could move up the stem, as well as down (Jacobs, 1952).

Since Coleus had also been used by some of the researchers who reported strictly downward differentiation of xylem cells, the unexpected upward transport of auxin seemed to destroy the correlation on which our research was based. But just before dropping the problem, we thought we should check the direction of xylem differentiation. When we collected regenerating stems every day during the regenerating period, we found that xylem differentiation was not strictly downward either: shortly before the downward differentiating strands reached the still intact portion of the xylem strand, short files of xylem cells would regenerate up to meet them. Thus, the correlation between auxin movement and xylem regeneration was even closer than the literature had originally suggested.

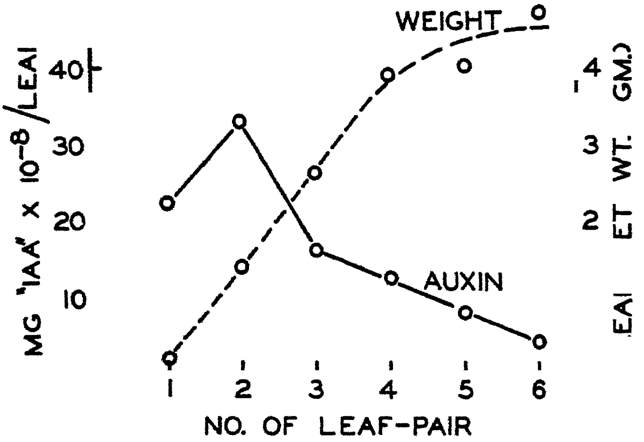


FIGURE 1. Amounts of auxin diffusing out of Coleus leaves of different positions and sizes, to show the relative amounts coming from different leaves.

If leaves were cut off (removing sources of auxin), there was a decrease in amount of xylem regenerated. If synthetic auxin, in the form of indoleacetic acid, were added to plants with leaves removed, the amount of xylem increased again.

These qualitative results support the interpretation that auxin formed in the leaves is the normal limiting factor for the differentiation of xylem cells during the regeneration of a xylem strand. When leaves are present as sources of auxin both above and below the regenerating internode (as in the normal condition), most of the regeneration is downward, with a smaller upward component, thus paralleling the ability of the stem to transport auxin (Jacobs, 1952).

Let us turn now to the quantitative relations.

QUANTITATIVE RESULTS

For this work, we used rigorously standardized material from a clonal stock of Coleus, thereby drastically reducing the variability. In addition, the samples were large in terms of most research on plant anatomy, and statistical tests of significance were routinely used.

Auxin-transport and xylem regeneration. When matched sections are cut from the standard internodes, and a concentration of synthetic indoleacetic acid slightly above the transport-capacity is added at either the top or bottom of the section, the amount of auxin collected at the other end is 65 units for "downward" (basipetal) transport, 20 units for "upward" (acropetal) transport. (A unit of auxin, as used here, represents 1×10^{-8} mg. IAA.) That is, when the same amount of auxin is available, more than three times as much can be transported basipetally as acropetally (Jacobs, 1954).

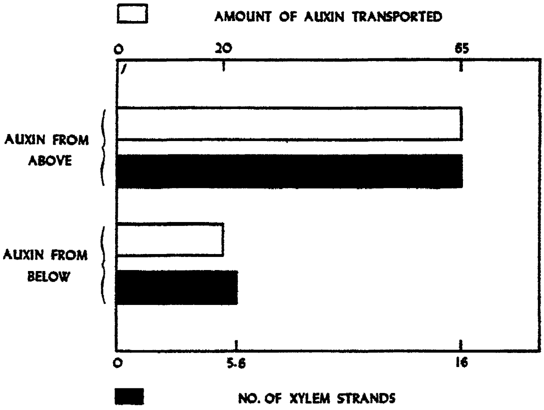


FIGURE 2. Relations between amount of auxin transported in isolated sections and the number of xylem strands regenerated in the plant when leaves above or below are excised.

Now let us see how the removal of the normally produced auxin supply affects the amount of xylem regenerated. This we can achieve by excising all the leaves either above or below the regenerating internode. If all the leaves above the wound are left on, an average of 16 strands of xylem are formed. (The number is not changed by removing the leaves below the wound.) If, on the contrary, the leaves above the wound are cut off while the leaves below the wound are left intact (thus leaving a source of acropetally moving auxin), then the number of xylem strands regenerated drops to an average of 5.6 strands. Figure 2 shows these results as they relate to the capacity of the stems for auxin transport in a basipetal vs. acropetal direction. It is obvious that there is a surprisingly exact parallel. The amount of xylem regenerated is proportional to the transport-capacity of the internode for auxin (Jacobs, 1954). In other words, it looks as if auxin is the limiting factor for xylem differentiation, but how much of it actually gets to the wound site seems to be controlled by the amount of auxin that the stem can transport.

Rate of auxin-production. Since the level of production is usually presumed to control how much auxin is available for auxin-controlled processes, it is now of interest to compare the normal level of production by the leaves with the transport-capacity of the stems for auxin.

Theoretically, there are three possibilities:

1) The level of production could be more than the transport-capacity;
2) the level of production could more or less equal the transport-capacity;
3) the level of production could be substantially less than the transport-capacity.

The third possibility we can immediately discard, since we would not have obtained the observed correlation between transport-capacity and xylem regeneration if the leaves were normally producing *less* auxin than the transport-capacity. So, production equals, or is larger than, the transport-capacity of the stem. Which is it? We can get evidence this way: if the leaves are producing auxin at a rate just matching the transport-capacity of the stem, then removing part of the auxin supply (as by cutting off only one leaf) should give a decrease in the amount of xylem which exactly and completely reflects the amount of auxin removed (as in the bottom half of fig. 3). But, on the other hand, if production is greater than transport-capacity, then removing one leaf will not give an exactly corresponding decrease in the amount of xylem regenerated, because part or all of that removal will be "used up" in just getting production down to the level of what the stem can transport (top half of fig. 3).

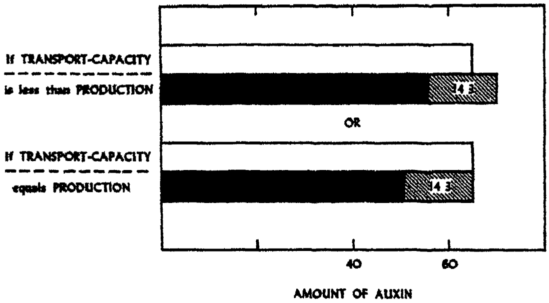
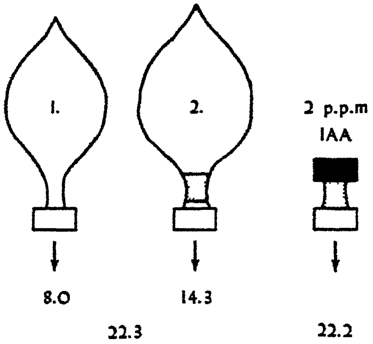


FIGURE 3. Graph illustrating the two possible effects which might be expected when part of the distal auxin supply is removed (by excising leaf 2 with its production of 14.3 units of "IAA" per hr.).

The amounts of auxin produced by the leaves just above the wounded internode are shown in figure 4. The leaf immediately above the wound (Leaf 2) produces an average of 14.3 units of "indoleacetic acid" per hour; the next leaf above that (Leaf 1) produces 8.0 units per hour. Now we need a quantitative measure of how much auxin gets into the plant when we add a known concentration of synthetic auxin in the form of indoleacetic acid. The method shown to the right of figure 4 was devised as a way of determining the physiologically significant amount of added indoleacetic acid that is transported from the site of application. Sections were cut from the leaf stalks of the standardized No. 2 leaves, indoleacetic acid was added to the leaf-blade (i.e., distal) end of those excised sections, and a block of agar was placed on the stem-end to collect auxin, as with the leaves to the left. As figure 4 shows, the addition of 2 p.p.m. of indoleacetic acid to the distal end gives 22.2 units per hour at the proximal end—an amount exactly the same as the combined production of leaves 1 and 2.



AUXIN FROM COLEUS LEAVES

FIGURE 4. The average amounts of auxin obtained per hour from No. 1 and No. 2 Coleus leaves and from a petiole section from No. 2 leaf which was capped distally with 2 p.p.m. indoleacetic acid.

If we cut off all the distal leaves (i.e., leaves No. 1 and 2, plus the 4-5 pairs of still smaller leaves in the apical bud), then add through the leaf stalk of leaf No. 2 this 2 p.p.m. of synthetic indoleacetic acid, providing 22 units of physiologically significant auxin, a marked increase is seen in the number of xylem strands regenerated. The increase is 35 per cent of the number of strands regenerating in the control plants with all leaves on.

If production equals transport-capacity, excising leaves 1 and 2, with their 22 units of auxin, should give a *decrease* of 35 per cent in the number of xylem strands. Figure 5 shows that this is exactly the case.

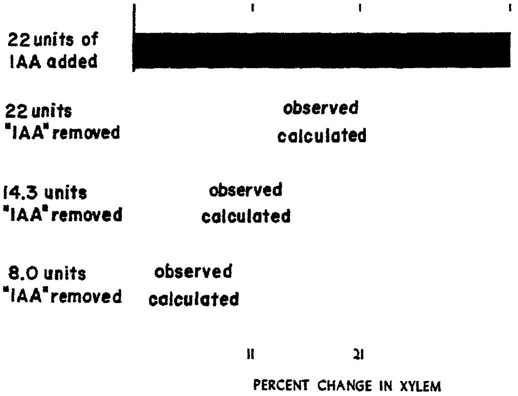


FIGURE 5. Graph showing effects on number of xylem strands regenerated per week of adding synthetic IAA, or of excising leaves 2 and/or leaves 1 (producing 14.3 and 8.0 units of "IAA" per hr., respectively). The data are given as per cent of strands regenerating in control plants with all leaves intact.

The bottom part of figure 5 also shows that the expected relations are found in other experiments where only leaf 2 is removed (with its 14.3 units of auxin) or where the 8.0 units from leaf 1 are removed (cf. Jacobs, 1956, for details).

These experiments, then, confirm the hypothesis that the leaves produce auxin at a level which normally just matches the transport-capacity of the stem below them. In other words, we cannot say that either auxin production or transport-capacity is *the* normal limiting factor for the differentiation of these xylem cells; normally they are both at the same level.

Auxin and non-regenerative xylem differentiation. When considering the evidence given above that auxin normally controls xylem differentiation during regeneration, one naturally wonders whether a similar relationship holds between auxin and xylem in the normal, non-regenerative development. As we have already seen (fig. 1), leaves of different sizes (and ages) produce different amounts of auxin. Leaves of the size and age which we have arbitrarily named "Leaf No. 2" produce auxin at the fastest rate.

If auxin is the limiting factor for xylem differentiation during *ordinary* development, then the number of xylem cells formed at the base of these different sized leaves should vary directly with the auxin production of the leaves.

Such counts were made recently by Paul Green. When the rate of formation of xylem cells formed at the base of the petioles was plotted against leaf size, the graph parallels the auxin curve shown in figure 1.

Thus, this first check supports the hypothesis that the differentiation of xylem during normal development is also limited by auxin from the leaves.

DISCUSSION AND SUMMARY

The most obvious conclusion from these experiments is that auxin is the normal limiting factor for the differentiation of xylem cells, the evidence being particularly extensive and quantitative with respect to xylem differentiation during regeneration of a severed vascular strand. The auxin controlling xylem differentiation is produced in the leaves and subsequently transported to the site of regeneration. The *entire* effect of the leaves in causing xylem regeneration is quantitatively explicable in terms of their production of auxin (when calculated as "indoleacetic acid"—cf. Jacobs, 1956, for further details).

The amount of auxin available for xylem regeneration is subject to control by both the amount produced in the leaves and the amount which the stem can transport. These two, transport-capacity and production, normally match, thus providing a "double check" on the auxin levels in the plant. (For a further discussion of the possible significance of the "double check" control, see Jacobs, 1956.)

A final point concerns the histochemical nature of auxin action. Auxin was first investigated, and is most widely known, as a hormone affecting growth. Most of the effects subsequently shown to be due to auxin application are also growth-promoting effects. This makes the clear relationship of auxin to xylem differentiation all the more puzzling, since apparently little or no growth of the parenchyma cells occurs prior to their transformation into xylem—at most a division or two precedes direct differentiation (Sinnott and Bloch, 1945). The most obvious change in these cells as they differentiate into xylem is the laying down of a thick secondary wall inside the thinner primary wall. This fact seems particularly significant when one remembers that auxin has an unusual position from the viewpoint of comparative biochemistry: unlike so many cell metabolites, it seems to have high activity only in plants—that is, among the organisms with cell walls. And the only group of plants where there is some question about the normal participation of auxin in growth is the fungi, plants with chitinous rather than cellulosic walls. These facts suggest that in its effects on both growth and xylem differentiation auxin is acting primarily on the cell wall.

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THE ORIGIN OF THE CULTIVATED CUCURBITA[1]

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INTRODUCTION

A symposium dedicated to Dr. Liberty Hyde Bailey is an appropriate occasion to discuss the Cucurbita. As a youth Dr. Bailey was fascinated by these, "marvels of the vegetable world," as he termed them. A sustained interest in this group of plants is suggested by his record of published research relating to the Cucurbita, commencing in 1890 and terminating in his monograph, "Species of Cucurbita," which appeared in 1943. In the intervening years, about one-half dozen papers were devoted to squashes, pumpkins and gourds. Some of Dr. Bailey's observations on these lusty, rapidly growing vines, with an apparently modest root system make interesting reading today. For example, "One spring I planted a single seed of a gourd in my greenhouse. It covered a long bench, ran out the ventilators at the top of the house, down the roof on the ground, full 100 feet by the time frost overtook it." Commenting on his experience of over 40 years with pumpkins, squashes and gourds he stated: "More than 1000 experiments were made, including several hundred hand crosses, and probably as many as 2000 nondescript forms of pumpkins and squashes were produced. The extent of the effort terminated itself, for the results were too voluminous to publish in full."

Except for that of corn, the archeological record of the cultivated Cucurbita is more abundantly documented than that of any other crop plant. Peduncles, rinds or shells, and seeds make excellent archeological subjects. The earliest archeological record from South America is that of *C. moschata* and *C. ficifolia* from Huaca Prieta, a site along the arid coastal region of Peru (Whitaker and Bird, 1949). This site has been dated by conventional methods as about 3000 B.C. In North America, the earliest record of Cucurbita is from the Ocampo Caves near Tamaulipas, Mexico (Whitaker, et al, in press). At the lower levels of this site, dated as about 3000 B.C., stems, rinds and seed of *C. pepo* have been recovered.

The American origin of all species of the cultivated Cucurbita is now established beyond reasonable doubt (Whitaker, 1947). Some data suggest that Central America and southern Mexico may have been the center of origin and domestication of this group (Vavilov, 1931). At any rate this would seem to be a likely area to search for evidence of this nature.

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[1] Presented at a Symposium on "The taxonomy of cultivated plants" dedicated to Dr. Liberty Hyde Bailey at a Joint Meeting of the American Society of Plant Taxonomists, American Society for Horticultural Science, American Society of Naturalists, Genetics Society of America and The Society for the Study of Evolution at East Lansing, Michigan, September 7, 1955.

The cultivated Cucurbita have much to offer the student interested in the origins of crop plants. In spite of their general similarity, five[2] species can be distinguished on the basis of their morphological characters. No single character can be used to separate all the species, and there are many overlapping characters. However, when good herbarium specimens, along with seeds and a photograph of the fruit, are present for examination, the specimens can be assigned to a particular species with a great deal of precision.

All species of Cucurbita examined have 20 pairs of chromosomes. The chromosomes are small and are not easily manipulated by the usual methods. For these reasons it has been necessary to develop special techniques adapted to this material (McGoldrick, Bohn and Whitaker, 1954).

The compatibility relationships of the several species have been investigated to the point where the overall picture is becoming increasingly clear. With one exception, interspecific matings produce sterile F_1 progenies and for the most part backcrosses to either parent are either sterile or sparingly fertile (Whitaker and Bohn, 1950, and unpublished data). The data from interspecific crosses suggest that each species has built up a system of sterility barriers capable of preventing gene flow between them, hence permitting the several species to maintain their identity even though some of them have been grown side by side for centuries. Nothing is known about the physiological basis of the sterility barriers, although there are a few experiments that indicate that in some species foreign pollen does not grow rapidly enough on the stigma and in the style of the recipient to effect fertilization. The diminutive embryos obtained in most interspecific crosses suggest that faulty endosperm development may be responsible for the small, starved embryos.

EXPERIMENTAL RESULTS

Description and cross-compatibility relationships of C. lundelliana. After several non-productive attempts to hybridize the cultivated species with a number of annual and perennial wild species, seed from four collections of the PETEN GOURD, *Cucurbita lundelliana* Bailey, was received in 1952. This species is reported to be widespread in Central America, notably in southeastern Mexico (Campeche), Guatemala and British Honduras (Bailey, 1943). *C. lundelliana* is an attractive annual with relatively thick, fleshy roots. The stems are slender and long-running and

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[2] The five cultivated species with the common names of their forms are:

annual {
C. pepo L.—summer squash, a few winter squash, pumpkin, ornamental gourd
C. moschata Duch.—winter squash, pumpkin
C. mixta Pang.—winter squash, pumpkin, cushaw
C. maxima Duch.—winter squash, pumpkin

perennial { *C. ficifolia* Bouché—fig-leaf gourd, Malabar gourd root at each node. Typical leaves are deeply 5-lobed, dull green, thin and softly hirsute, with entire margins; the petiole is slender with soft hairs. The staminate flowers are large and handsome on long, slender pedicels; the pistillate flowers are similar in appearance, but with shorter, stouter pedicels. The fruit is a small (7.5 × 7.0 cm), green, round, hard-shelled gourd, slightly tapered at the stem end, with bitter, stringy, greenish-white flesh. The peduncle is sharply angled and furrowed, but not flared at the fruit attachment. The seeds are small (10 × 5 mm), ash-grey, with a broad heavily sculptured margin, tapering to an acute base.

The significant geographical distribution of *Cucurbita lundelliana* suggested at once that its relationship to the cultivated species might be worth investigation. Accordingly, pistillate flowers of *C. lundelliana* were pollinated with staminate flowers from each of the five cultivated species. Surprisingly the pistillate flowers of *C. lundelliana* set fruits readily regardless of the pollen source. The F_1 and the progenies of the backcross to each parent from three of these matings have been grown and studied (*C. lundelliana* × *C. maxima*, *C. lundelliana* × *C. moschata*, and *C. lundelliana* × *C. ficifolia*). The other two (*C. lundelliana* × *C. mixta* and *C. lundelliana* × *C. pepo*) have produced fruits with abundant seeds, but the embryos are small. Special techniques, probably embryo culture, will be required to produce F_1 plants from this material.

At this time it is not proposed to examine the behavior of each cross and the resulting progeny in detail, but rather to discuss the significance of the observations as they are related to the origin of the cultivated group.

Pollen fertility of the F_1 hybrids. There is a wide variation in pollen abortion of the F_1 hybrids from the various crosses as indicated by the percentage of stainable pollen in aceto-carmine smears. This variability depends upon the species used as a pollen parent with *Cucurbita lundelliana*. When *C. moschata* is used, 42 per cent of the pollen from the F_1 plants is normal; 17 per cent in the cross with *C. maxima*; and only 15 per cent when *C. ficifolia* is used as one parent. In general, more plump seed per fruit was obtained from F_1 plants with the highest percentage of stainable pollen, as would be expected.

Cytology of the F_1 hybrids. A thorough study of meiosis in the three species crosses that produced F_1 hybrids has not been completed. However, enough has been done to indicate that meiosis in each is uncommonly regular. For the most part there are 19 or 20 bivalents; in a few cells an occasional quadrivalent and as many as 4 unpaired chromosomes in some cells may occur. In the F_1 from the cross *C. lundelliana* × *C. maxima* bridge-fragment configurations were fairly common.

DISCUSSION AND CONCLUSIONS

The pattern formed by these compatibility relationships is sufficiently unusual to deserve comment. Here we have five cultivated species, rather similar in their gross morphological characters and for the most part cross-incompatible among themselves, but cross-compatible with a non-cultivated species. Furthermore, the F_1 hybrids from three of these species crosses are self-fertile and cross-fertile with each parent.

The results of the crosses are indicative of the genetic affinities between the cultivated species. On the basis of their mating behavior with *C. lundelliana* they separate sharply into two groups. The first group consists of three species (*C. moschata*, *C. maxima* and *C. ficifolia*). In this group, matings produce seeds with normal embryos and the F_1 plants are self-fertile and cross-fertile with either parent. In the second group of two species (*C. mixta* and *C. pepo*) the F_1 seeds produce small embryos that do not emerge from the seed coats under the usual conditions for good germination.

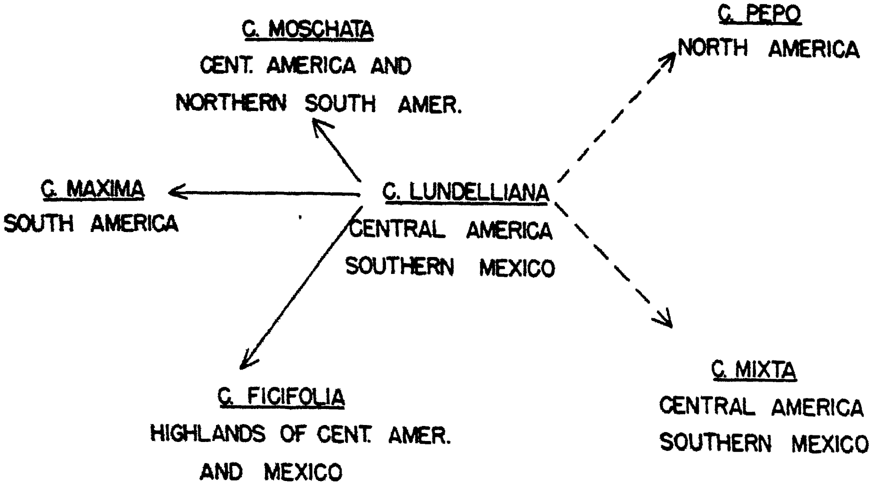

FIGURE 1. The geographical interrelationships of the five cultivated species of *Cucurbita* with *C. lundelliana*. (See further explanation in the text.) The solid lines indicate that F_1 hybrids with *C. lundelliana* produce seeds with normal embryos; the broken lines indicate that seeds with small sub-normal embryos are produced.

This separation into two groups suggested by the results of the matings with *Cucurbita lundelliana* is strengthened by the breeding behavior of the five cultivated species among themselves. The hybrids obtained from crosses between *C. maxima* and *C. pepo* and *C. maxima* and *C. mixta* resemble one another in contrast to hybrids obtained by mating *C. maxima* with *C. moschata*, where the fruit from the F_1 hybrids is altogether different in appearance. Furthermore *C. maxima* crosses readily with *C. moschata*, producing partially fertile F_1 hybrids and self-fertile, stable amphidiploids. It crosses with *C. mixta* and *C. pepo* only with difficulty. The F_1 hybrids from these matings are nearly sterile, and the amphidiploids are unstable and sparingly fertile. *C. ficifolia* occupies a central position as it crosses with *C. maxima* in both directions and with *C. pepo* only when *C. ficifolia* is used as the female parent. However, the hybrids from these crosses are completely sterile (Weiling, 1955).

The geographic interrelationships of the five cultivated species with *C. lundelliana* are diagrammed in figure 1. At the extremes are *C. maxima* in South America and *C. pepo* in North America. In the central portion of the hemisphere are *C. moschata* in Central America and northern South America and *C. mixta* in Central America and southern Mexico. These are all lowland species. In the highlands of Central America and Mexico, *C. ficifolia* is found.

We assume that these studies have some practical value, possibly for the plant breeder. For instance, *C. lundelliana* might be utilized as a bridge to transfer certain desirable characters of *C. pepo* to *C. maxima*: for example, squash bug resistance. This transfer could probably be done at the diploid level, and in all likelihood satisfactory fertility could be retained. Moreover, tolerance to powdery mildew and the thrifty, vigorous vine habit of *C. lundelliana* might be easily transferred to the cultivated species by appropriate breeding procedures.

The cross-compatibility of *C. lundelliana* with each of the cultivated species suggests that they were derived from a common gene pool or at the very least that they must have an identical complement of several genes. The sterility barriers that are effective in maintaining the identity of the cultivated forms are negated in matings with *C. lundelliana*. Evidently this species is the common denominator of the entire group. The data from these experiments lead one to suspect that the sterility barriers which operate effectively in the cultivated species are confined to this group and are probably not present in the non-cultivated members of the genus.

Does this mean that *Cucurbita lundelliana* may have been the ancestor of the cultivated group? Our studies have not progressed to the point where we can give an informed answer to this question. However, there are two pieces of evidence that support an affirmative answer: (1) the distribution of *C. lundelliana* in Central America and southern Mexico coincides with the hypothesis that the center of origin of the cultivated Cucurbita is to be sought in this general region; (2) the hybridization experiments indicate some measure of compatibility between *C. lundelliana* and each of the five species of cultivated Cucurbita.

SUMMARY

The five cultivated species of Cucurbita (*C. pepo*, *C. mixta*, *C. moschata*, *C. ficifolia* and *C. maxima*) are very much alike in their morphological characteristics, but they are for the most part cross-incompatible. They have apparently developed a sequence of sterility barriers that effectively prevent gene flow between them. In a series of interspecific matings, the five cultivated species are shown to be cross-compatible with a wild species, *C. lundelliana*, from southern Mexico and neighboring Cen-

tral America. Matings between *C. lundelliana* and *C. moschata*, *C. maxima* and *C. ficifolia* produced self-fertile F_1 plants that were cross-fertile with either parent. The other two matings (*C. lundelliana* × *C. mixta* and *C. lundelliana* × *C. pepo*) produced fruit, but the seeds had small embryos. The data from interspecific hybridization and those of geographical distribution suggest that *C. lundelliana* may have had an important role in the origin of the cultivated group.

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CRYPTIC COLORATION IN THE ANTHOPHILOUS MOTH *RHODODIPSA MASONI**

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Most Heliothidine moths are characterized by contrasting coloration, by association with one or few species of plants, and by cryptic resemblance to the flowers of these plants (Grote 1882; Bird 1923; Kwiat 1908; Wyatt 1938, 1941). Furthermore, the larvae of each species usually feed on the flowers and seeds of the same plant species on which the adults are characteristically found. Cockerell (1910) was the first to record the particularly striking resemblance of the adult *Rhododipsa masoni* J. B. Smith (Lepidoptera, Noctuidae, Heliothidini) to the inflorescence of the Composite, *Gaillardia aristata* Pursh, on which it always was found. He mentioned that the moth rests on the flower in such a way "that although the insect is brightly colored, it becomes practically invisible." The present paper is a quantitative study of this color relationship.

The authors wish to express their appreciation to Dr. C. L. Remington for his helpful criticism and discussion in the writing of this paper, and to Drs. E. B. Ford and P. M. Sheppard for their enthusiastic suggestions while in Colorado. We want to thank Dr. J. W. Marr of the Institute of Arctic and Alpine Ecology, University of Colorado, for arranging our research facilities at Science Lodge. We also appreciate the kind help of Messrs. J. G. Coutsis, D. Eff, R. R. McElvare, and A. K. Wyatt. This work was carried out as an adjunct to research in Colorado aided by a grant from the Sigma Xi-RESA Research Fund.

All recorded observations were made between 15 June and 10 July 1955, in the foothill and montane zones of the Rocky Mountains in Boulder County, Colorado. The moths were first observed by us on the flowers of *G. aristata* on 20 June 1954, at an altitude of approximately 8500 feet, along a road built on top of the old Denver-Rio Grande Railroad bed just west of Sugar Loaf Mountain. In 1955, observations were made along this road, the road from Boulder Canyon to Sugar Loaf Mountain, the road from Colorado Highway 160 to Gold Hill, and in Buckingham Park at the bottom of Left Hand Canyon. The approximate altitudinal range of all moths found was 5900 feet in Buckingham Park to 8700 feet along the road to Gold Hill. Altitudes were taken from the 1904 U.S.G.S. Boulder Quadrangle Map. The moths were found only where blooming *G. aristata* also occurred.

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*This paper is dedicated to Professor Alexander Petrunkevitch on the occasion of his 80th birthday.

The moth is conspicuously colored. Both head and thorax are Cadmium Yellow which contrasts strikingly with the predominantly Ox-blood Red forewings. The color of the hindwings (Dark Vinaceous-Brown) is unimportant here since they are covered by the forewings while the moth is feeding and when it is at rest. (These colors were determined by comparison to Ridgway's (1912) color tables.) The inflorescence of *Gaillardia aristata* is colored in the following general manner: the ray florets are yellow; red disk florets occur in a concentric band just inside the ray florets, and yellow disk florets compose the center of the flower. Thus from the periphery to the center, the flower is yellow, then red, then yellow. The size, color, and color proportions of any one flower vary throughout development, and even at any one stage of development there is variation from flower to flower. The yellow and red colors of the moth and the flower are very similar, but not identical. The accompanying photograph (figure 1) shows a moth on a flower which is at an early stage of development.

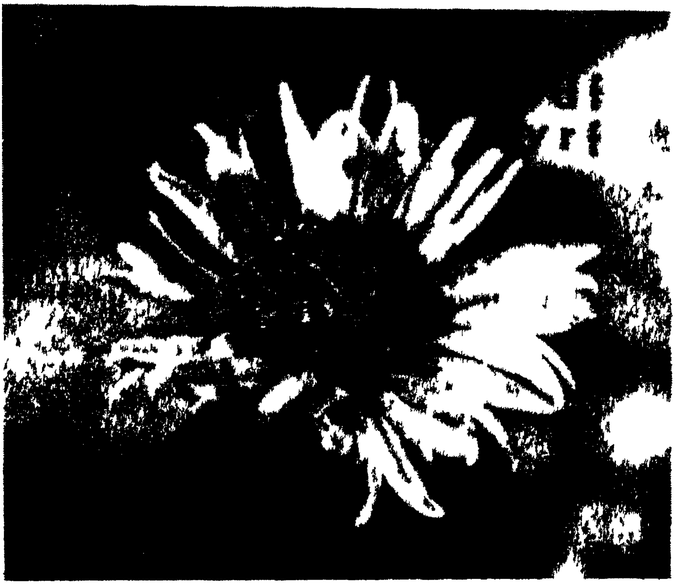

FIGURE 1. *Rhododipsa masoni* adult at rest on inflorescence of *Gaillardia aristata* in the typical "head-out, inconspicuous" position. Approx. 1.5 natural size. For the moth, rfw = red forewing; yt = yellow thorax; for the Composite flower, yrf = yellow ray floret; rdf = red disk florets; ydf = yellow disk florets. (Photograph by the authors).

It was noted that a moth could be either conspicuous or inconspicuous on a *G. aristata* flower depending on how it was oriented. It was inconspicuous if its yellow anterior part was over either the yellow ray florets ("head-out," see figure 2, position 1), or over the yellow central part of the disk ("head-in," see figure 2, position 2). In these two positions the colors of the moth and flower blended, and the moth was much less conspicuous than when it was oriented "sideways" (see figure 2, position 3). In the "sideways" position, the yellow anterior part contrasted with the underlying red disk florets, thereby making the moth conspicuous. However, in a few cases the conspicuous or inconspicuous condition of the moth was not in agreement with the orientations just described (see figure 2, positions 4 and 5). Therefore each moth was recorded both according to its class of orientation, *i.e.* "head-in," "head-out," or "sideways," and according to whether the colors of the moth contrasted or harmonized with those of the Gaillardia flower, *i.e.* "conspicuous" or "inconspicuous" (see table). Moths not on the upper surface of *G. aristata* flowers were recorded according to the substrate on which they were found. Although considerable time was spent observing other plant species in the same areas, no moths were found on any except those listed in the table. It is assumed that each observation represented a different moth. This assump-

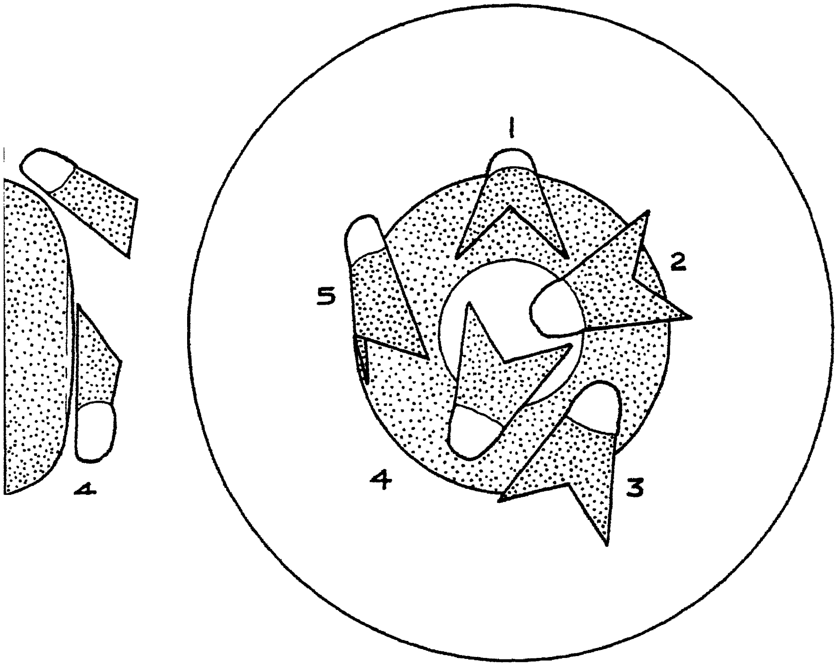

FIGURE 2. On right: Top view. Diagrammatic representation of moths on an inflorescence to indicate the relationship of various positions to conspicuousness. Stippled area = red, open area = yellow. 1: head-out, inconspicuous; 2: head-in, inconspicuous; 3: sideways, conspicuous; 4: head-out, conspicuous; 5: sideways, inconspicuous. On left: Side view of same. For clarity, only moths in positions 1 and 4 are shown.

TABLE

DISTRIBUTION AND APPEARANCE OF 26 *RHODODIPSA MASONI* ADULTS ON VARIOUS SUBSTRATES DURING DAYTIME.

On upper surface of *Gaillardia aristata* flower						On other substrates			
Head-out		Head-in		Sideways		Underside *G. aristata* flower	Green leaf *G. aristata*	Green leaf *Alnus tenuifolia*	Bud *Cirsium undulatum*
Incon.	Con.	Incon.	Con.	Incon.	Con.				
14	2	2	0	1	2	2	1	1	1
Total Inconspicuous 17									
Total Conspicuous 4									

tion seems justified, because the records were taken over a wide area at different times and often were made simultaneously by two observers.

On the basis of random scattering, one would expect to find an equal number of moths on the upper surface of *G. aristata* flowers and on each of the other four substrates included in the table. However, 23 out of 28 moths were observed on top of the flowers (an additional 2 moths not included in the table flew off the upper surface of *G. aristata* flowers, raising the total observations from 26 to 28). The probability that this distribution occurred by chance alone is less than .001. Thus it is clear that *R. masoni* is associated with the upper surface of *G. aristata* flowers. A comparison of the moths which were inconspicuous (17) to those which were conspicuous (4) indicates that the number inconspicuous deviates significantly from the number expected on the basis of a .5–.5 distribution ($P < .005$). This supports the contention that the moth is in a cryptic position when on the upper surface of *G. aristata* flowers. If the 6 positions of the moths on the upper surface of the flowers are compared, it is seen that the distribution in these columns deviates significantly from the hypothesis that a moth has an equal chance of acquiring any 1 of the 6 positions when it is on the upper surface ($P < .001$). However, if the first column ("head-out, inconspicuous") is excluded and all the remaining columns compared, it is seen that the moths are randomly scattered throughout the 9 columns. Thus, a non-random distribution of the moths results from the predominant number of moths in the "head-out, inconspicuous" position on the flower.

DISCUSSION

Seen as a museum specimen, this small moth with its bright contrasting colors might seem a protected species advertising its noxious qualities through its bright warning coloration. However, Cockerell's note (1910) and the data now presented clearly indicate that the coloration of *R. masoni* is, in fact, cryptic due to its intimate relationship with the brightly colored flowers of a specific Composite plant. The details of this cryptic coloration are exemplified by a comparison of the visual textures of the flower and the moth. Each flower is composed of numerous units which together present a mosaic appearance; a similar effect is produced by the mottled forewing pattern of the moth. This fact, plus the red-yellow color division of the moth, correlated with its typical orientation produces a remarkable resemblance to the various parts of the flower (see figure 1).

It was pointed out above that a moth could be cryptic in either a "head-out" or a "head-in" position, but the data indicated a preponderance of the moths in the "head-out" position. This seems to be correlated with the mode of feeding of the moths and with the blooming sequence of the inflorescence. The florets of the disk mature in sequence towards the center. The moths were observed feeding radially on the mature peripheral florets of the disk, while at the same time the central florets were immature and offered no source of nectar. At the end of feeding, each moth usually maintained the "head-out" orientation and settled down with its yellow anterior region adjacent to or over the yellow ray florets and its red wings over the red disk florets. Although the possibility exists for moths to feed in a "head-in" orientation, this is unlikely because the ray florets were observed to be mechanically unsuited as a feeding platform. Thus the typical centrifugal orientation of the moth appears to be related to its mode of feeding which in turn seems to be determined by the centripetal blooming sequence of the individual florets of the Composite. The data presented suggest that the habit of feeding head-out on the disk of the Gaillardia flower preceded the evolution of the bicoloration of the moth. It seems likely that natural selection established the bicoloration of the moth for better cryptic resemblance because its normal feeding orientation on the Gaillardia flower placed it on a bicolored background.

SUMMARY

1. Data are presented which show the intimate relationship of a bicolored moth with a similarly bicolored Composite flower.
2. The typical orientation of the moth on the flower is such that its colors blend with those of the flower; the moth therefore possesses cryptic coloration on the flower.
3. This color orientation of the moth arises from the way in which the moth feeds on the Composite flower and this feeding position in turn is determined by the blooming sequence of the individual florets.
4. It seems likely that selection favored evolution of the bicoloration of the moth because its normal feeding orientation on the Gaillardia flower placed it on a bicolored background.

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A NUTRITION-INHIBITION HYPOTHESIS OF PATHOGENICITY

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The categories of parasitism may be determined by the type of response exhibited by the host to an entry or to the presence of the parasite. Pathogenicity may be distinguished from saprophytism and symbiosis in that the host is adversely affected by the parasite. It is possible for a given relationship to shift from one category to another under certain conditions. The successful invasion and subsequent extensive multiplication or metabolism in a host with its characteristic attendant reactions are the basis for considering a parasite to be pathogenic and simultaneously, the host to be susceptible. On the other hand, if the parasite fails either to proliferate or to metabolize extensively after invasion, this may reflect either the avirulence of the parasite or the resistance of the host or both. Consequently, pathogenicity must always be defined as an expression of a two component system: the host-parasite relationship. Such a definition which also applies to the other categories of parasitism is basically ecological, emphasizing the host as an environment for the parasite.

Pathogenicity as it concerns the parasite assumes that the parasite is capable of utilizing the host environment as a growth medium and that the parasite can overcome the total defense mechanisms of the host. It should be noted that two problems are involved: the ability of the parasite to proliferate or to metabolize extensively and the mechanism(s) whereby the parasite damages either the host *in toto* or certain cells or tissues of the host. The host may be resistant either if it does not provide a suitable growth medium for the parasite or if the host presents defense mechanisms which prevent, inhibit, or restrict the potential proliferation or metabolism of the parasite. These considerations have led to the formulation of a nutrition-inhibition hypothesis of pathogenicity. Lewis (1953) has presented a comparable hypothesis from a different point of view in his "balance hypothesis of parasitism."

The nutrition-inhibition hypothesis of pathogenicity involves the two environments in the host which directly affect the fate of an invading parasite: a nutritional environment and an inhibitory environment. If the nutritional environment is inadequate for the parasite, the latter can neither proliferate

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nor metabolize extensively and will not be virulent; if the nutritional environment is adequate for the parasite, the latter will be able to proliferate or to metabolize extensively but may or may not be virulent. An effective inhibitory environment may or may not also be present. In the absence of an effective inhibitory environment, a parasite in an adequate nutritional environment generally will be virulent; in the presence of an effective inhibitory environment, the parasite will not be virulent even though the nutritional environment is adequate. Of the four possible combinations of host environments, only the adequate nutrition-ineffective inhibition environment results in virulence; the other environments result in avirulence. The antigenic, morphological, and enzymatic characteristics of the parasite add complications to be discussed later.

NUTRITIONAL REQUIREMENTS OF THE PARASITE

The role of nutrition in the host-parasite (pathogen) relationship will be referred to the nutritional requirements of the parasite as they may or may not be satisfied by the host environment. The parasite must get all of its nutritional requirements from the host either at the site of inoculation or of localization. Many pathogens have nutritional requirements beyond the basic need for certain elements and an energy source, indicating that they no longer synthesize certain materials such as amino acids, vitamins, growth factors, *etc.* The relationship between the host as a growth medium and the specific nutritional requirements of the parasite, including animal and plant pathogens, has been subjected to direct study only comparatively recently (Bacon, Burrows, and Yates, 1950, 1951; Garber, Hackett, and Franklin, 1952; Garber, 1954; Keitt and Boone, 1954).

Considerable effort has been expended in clarifying the role of such inhibitory environments as the humoral and cellular defense mechanisms of animal hosts. Although varieties and species of plants resistant to certain parasites have long been known, the mechanisms conferring resistance on these hosts are not yet adequately understood. The defense mechanisms of the host constitute the potentially inhibitory environment which the parasite must overcome if it is to be pathogenic. It should be emphasized that a specific defense mechanism effective for a specific parasite may be ineffective for another parasite. Also, defense mechanisms need not be absolute in their effectiveness.

Any hypothesis concerning pathogenicity must also consider within its framework non-pathogenic as well as pathogenic species or strains. Although the nutrition-inhibition hypothesis provides three distinct situations resulting in avirulence, it may be technically difficult to assign a specific example of avirulence to a specific situation. Furthermore, the parasite may alter its immediate environment within the host. For example, the animal host may produce antibodies or antitoxins as the parasite is proliferating or metabolizing; the plant host may produce structures limiting the spread of the inoculum. The nutritional-inhibitory environments in the host may be influenced by the host's environment. Consequently, examples of the possible situations indicated by the nutrition-inhibition hypothesis may be unique in their simplicity. These examples, however, may serve as models for future investigations.

VIRULENCE

A parasite confronted with an adequate nutritional environment and an ineffective inhibitory environment generally will be virulent. If the parasite proliferates or metabolizes extensively in a host, the host must be providing all the nutrilites required by the parasite; a susceptible host *ipso facto* presents an ineffective inhibitory environment. Experimental evidence indicating that the host is indeed providing specific nutrilites has come from studies using biochemical mutants of animal and plant pathogens (*Salmonella typhosa*, Bacon *et al.*, 1950, 1951; *Klebsiella pneumoniae*, Garber *et al.*, 1952; *Erwinia aroideae*, Garber, 1954; *Venturia inaequalis*, Keitt and Boone, 1954; *Agrobacterium tumefaciens*, Garber *et al.*, 1955). These experiments employed biochemical mutants with specific requirements for an amino acid, vitamin, growth factor, purine, or pyrimidine. For example, the virulence of biochemical mutants of *K. pneumoniae* was determined by inoculating mice intraperitoneally or intranasally. Mutants requiring threonine, tyrosine, methionine, leucine, histidine, or uracil (pyrimidine) retained their virulence (Table 1). Cultures obtained from the peritoneal fluid of animals succumbing after an intraperitoneal inoculation and from animals succumbing after an intranasal inoculation were tested to determine whether the specific nutritional requirement was still present. In all cases, the recovered cultures were still mutant, indicating that reversion to prototrophy (the parental type) had not occurred in the host. Finally, peritoneal

TABLE 1

RELATION BETWEEN VIRULENCE AND NUTRITIONAL REQUIREMENTS OF BIOCHEMICAL MUTANTS OF *KLEBSIELLA PNEUMONIAE* INOCULATED INTO MICE EITHER INTRAPERITONEALLY OR INTRANASALLY (GARBER *ET AL.*, 1952).

		Route of Inoculation			
Mutant	Need	Intraperitoneal		Intranasal	
		Dosage	Dead/Total	Dosage	Dead/Total
"wild"	none	1×10^4	9/10	3×10^5	9/10
34	threonine	1×10^4	10/10	3×10^5	10/10
2	tyrosine	1×10^4	10/10	3×10^5	10/10
40	methionine	1×10^4	9/10	3×10^5	10/10
27	leucine	1×10^4	9/10	3×10^5	8/10
17	histidine	1×10^4	10/10	3×10^5	10/10
22	uracil	3×10^5	4/5	7×10^4	2/5
2711	quanine	3×10^4	0/10	8×10^5	0/10
58	adenine	1×10^5	0/10	3×10^6	0/10
58–01	none	1×10^4	9/10	3×10^5	9/10
58–02	none	1×10^4	6/10	3×10^5	9/10

fluid from the mouse was tested for its ability to satisfy the nutritional requirements of these virulent, biochemical mutants and in each case, the nutritional requirement was satisfied. Consequently, a direct relationship between the nutritional requirements of these virulent, biochemical mutants and the availability of the specific, required nutrilite in the host either at the site of inoculation or of localization was established.

It has been possible to demonstrate an apparent increase in virulence by supplying either "preformed" materials or a greater amount of the substrate presumably required by the parasite in the host. Bacon *et al.* (1951) injected purine into the peritoneal cavity of the mouse along with the parental strain of *S. typhosa* which does not require exogenous purine and observed a significant increase in virulence. Berry and Mitchell (1953) introduced Krebs cycle poisons along with *Salmonella typhimurium* into the peritoneal cavity of the mouse and increased the virulence of the pathogen, presumably by increasing the concentration of the energy source available to the pathogen.

If the parasite cannot proliferate or metabolize extensively in the host, it cannot be virulent. Avirulence may result either from an inadequate supply of required nutrilites or the presence of an effective inhibitory environment. In some instances, it has been possible to distinguish between these alternatives. Avirulence may also result from both situations occurring together.

TABLE 2

THE EFFECT OF INTRAPERITONEAL INOCULATIONS OF THE COMPLEX PURINE-LESS MUTANT, 27139, OF *KLEBSIELLA PNEUMONIAE* AND EITHER HYPOXANTHINE OR A PURINE POOL* (GARBER *ET AL.*, 1952).

Expt.	Treatment	Total Dosage	Dead/Total
I	27139	1.3×10^5	0/10
	hypoxanthine	50 mg.	0/10
	27139 plus	1.3×10^5	
	hypoxanthine	50 mg.	3/10
II	27139	8×10^6	0/10
	purine pool*	24 mg.	0/10
	27139 plus	8×10^6	
	purine pool*	24 mg.	10/10

*15 mg. hypoxanthine, 3 mg. xanthine, 3 mg. adenine, 3 mg. guanine.

The greatly diminished virulence or avirulence of purine-requiring mutants of *S. typhosa* (Bacon *et al.*, 1951), *K. pneumoniae* (Garber *et al.*, 1952), and *Pasteurella pestis* (Burrows, 1955) reflects an inadequate nutritional environment in the host, the mouse. Burrows (unpub.) noted that a purine-requiring mutant of *P. pestis* is likewise avirulent for the guinea pig and the rat. The purine-requiring mutants of *K. pneumoniae* (2711, 58) were avirulent for the mouse when inoculated either intraperitoneally or intranasally (Table 1). Peritoneal fluid from the mouse did not satisfy the nutritional requirements of these biochemical mutants. Prototrophic reversions

(58–01, 58–02) from a purine-requiring mutant were virulent when inoculated either intraperitoneally or intranasally (Table 1). Finally, introducing purine and a purine-requiring mutant into the peritoneal cavity of the mouse resulted in a display of virulence (Table 2). Cultures recovered from the peritoneal cavity and lungs of dead mice were mutant, indicating that reversions to prototrophy were not responsible for the death of the mice. Moribund mice were sacrificed and only mutant cultures were recovered.

AVIRULENCE AND NUTRITION

Experiments with plant pathogens have provided confirmatory evidence that an inadequate nutritional environment may result in avirulence (Garber, 1954; Keitt and Boone, 1954). For example, an arginine-requiring mutant of *E. aroideae*, a bacterial plant pathogen, was avirulent for the radish and the turnip when the cut surface of the fleshy storage organ was inoculated (Garber, 1954). If a block of tissue from the storage organ was added to a culture of this mutant in the minimal agar, no bacterial growth was observed around the tissue block (Fig. 1). However, if a homogenate of the entire storage organ was added as a drop to a culture of this mutant in the minimal agar, growth was observed around the drop. These observations indicated that the required nutrilite was present *in* the tissue in sufficient concen-

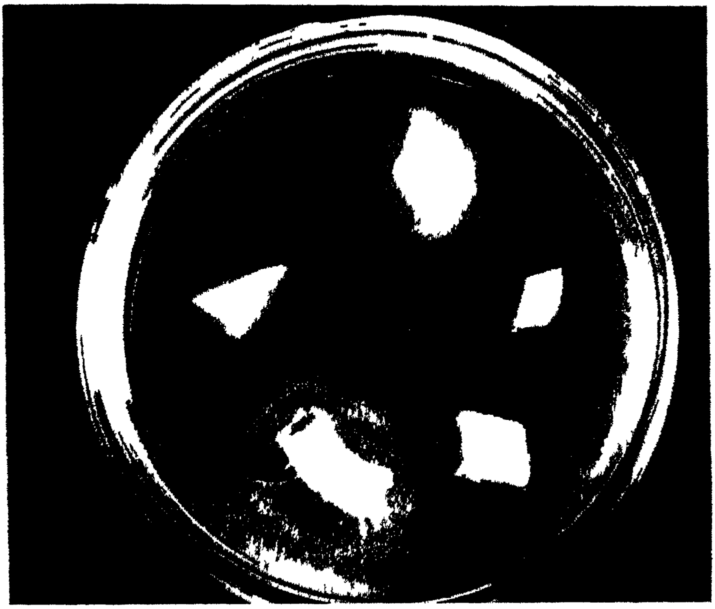

FIGURE 1. The response of an arginine-requiring mutant of *Erwinia aroideae* to blocks of storage organ tissue of the potato (susceptible), carrot (resistant), sweet potato (resistant), parsnip (susceptible), and radish (resistant). The potato tissue block is at 12 o'clock, the other tissue blocks occurring clockwise.

tration to support the growth of the mutant but the concentration of the required nutrilite either at the site of inoculation or at the surfaces of the tissue block was inadequate to support the growth of the mutant. On the other hand, the arginine-requiring mutant was virulent for the potato. Growth was observed around the block of potato tissue added to a culture of the mutant in minimal agar (Fig. 1).

Avirulent strains or species of pathogens may proliferate or metabolize for some time after invasion and then stop either proliferating or metabolizing. Although an effective inhibitory mechanism may be assumed to be operating in the host, such observations may also reflect an inadequate nutritional environment. For example, an arginine-requiring mutant of *E. aroideae* was avirulent for the radish using a standardized procedure prior to inoculation (Garber, 1954). According to this procedure, mutant cells previously grown in nutrient broth supplemented with yeast extract were washed twice in 0.85 per cent saline prior to inoculation, a process lasting 2 hours. The following procedure was then adopted: the cells were washed once and then suspended in 0.85 per cent saline, a process lasting 30 minutes, and immediately incubated at 27° C. Arginine could not be detected as carry-over from the broth in the saline suspensions of cells. Inocula were then extracted every 15 mins. for a virulence test on slices of radish. The inoculum taken just prior to incubation served as a control. The control cells and the cells incubated for 60 mins. were virulent; the cells taken at 75 mins. were weakly virulent; and the cells extracted at 90 to 120 mins. were avirulent (Garber, unpub.). These results suggest that an initial, endogenous stockpile of arginine or materials yielding arginine was diminishing until it was inadequate to support growth in the absence of an exogenous supply of the required nutrilite. It had already been established that the site of inoculation lacked an adequate supply of arginine to support a proliferating population of arginine-requiring cells of *E. aroideae* (Garber, 1954).

TABLE 3

SYNERGISTIC EFFECT OF PURINE-LESS AND PARA-AMINOBENZOIC ACID-LESS (PABA) MUTANTS OF *SALMONELLA TYPHOSA* INOCULATED INTRAPERITONEALLY INTO THE MOUSE (BACON *ET AL.*, 1951).

Total dose of organisms	Deaths out of 20 mice inoculated with		
	Purine-less mutant	PABA-less mutant	Purine-less and PABA-less mutants
$\times 10^{-6}$			
100	0	0	2
200	0	1	17
400	2	1	18
800	9	10	20

Bacon *et al.* (1951) have provided an interesting example of the virulence of a mixed culture of two biochemical mutants of *S. typhosa*, each mutant itself being relatively low in virulence for the mouse (Table 3). One mutant required purine; the other mutant required PABA. If suboptimal amounts of the required nutrilites were supplied to a mixed culture of these mutants *in vitro*, growth was normal and a heavy suspension of cells resulted. This observation indicated a syntrophic effect, *i.e.*, the purine-requiring mutant excretes during its metabolism a material satisfying the nutritional requirement of the mutant requiring PABA and the latter mutant excretes in turn a material satisfying the nutritional requirement of the mutant requiring purine (Davis, 1950). If the host provides suboptimal concentrations of either purine or PABA, the nutritional requirements of these biochemical mutants would then be satisfied not by the host but by the metabolic products of the mutants themselves.

TABLE 4

EFFECT OF DIETARY PARA-AMINOBENZOIC ACID (PABA) ON THE VIRULENCE OF A PABA-LESS MUTANT OF *SALMONELLA TYPHOSA* INOCULATED INTRAPERITONEALLY INTO MICE (BACON *ET AL.*, 1951).

Culture	PABA given on days*					Challenge	Deaths
	5	4	3	2	1	Dose × 10^{-6}	
PABA-less mutant	–	–	–	–	–	400	3
	–	–	–	–	–	100	0
	–	–	–	–	+	100	16
	–	–	+	+	+	100	18
	+	+	+	+	+	100	20
parental strain	–	–	–	–	–	40	12
	–	–	–	–	–	10	8
	–	–	–	–	+	10	9
	–	–	+	+	+	10	10
	+	+	+	+	+	10	3
	+	+	+	+	+	10	0

*20 mice per treatment, each given 2 ml. water or PABA solution (5 mg. per ml.) on the days prior to challenge as indicated: + = PABA, – = water.

A nutritionally inadequate environment for the parasite may become a nutritionally adequate environment by altering the host's diet. Bacon *et al.* (1951) have provided an interesting model relating host diet to the virulence of a mutant of *S. typhosa* requiring PABA. Mice were fed PABA in their diet for a specific number of days prior to an intraperitoneal inoculation either with the mutant requiring PABA or the parental strain not requiring exogenous PABA. This biochemical mutant usually displaying a greatly diminished virulence became increasingly virulent for mice getting PABA in their diet prior to inoculation. Supplementing the diet with PABA prior to inoculation with the parental strain had no significant effect on the virulence of the parental strain (Table 4).

It might seem that studies on the virulence of biochemical mutants of pathogenic microorganisms represent laboratory models without parallel in nature. Formal *et al.* (1954) have documented a case of greatly reduced virulence in *S. typhosa* which was the result of a spontaneous mutation *in vitro* to a requirement for purine. Furthermore, it is now clearly established that the virulence of certain species of Plasmodium is related to the availability of PABA and methionine in the host (reviewed by McKee, 1951). For example, the growth and proliferation of certain species of Plasmodium is retarded *in vivo* in animals which have been starved either during a malarial infection or at the time of infection. This situation is maintained as long as the host remains starved. Growth and proliferation of the plasmodia follow the restoration of an adequate diet or the addition of methionine and/or PABA to the diet.

INHIBITION MECHANISMS

Perhaps the most common and important situation resulting in avirulence concerns the host which presents an adequate nutritional environment and an effective inhibitory environment. Inhibitory mechanisms may include biological, chemical, and physical antimicrobial agents. An appreciation of the significance of an effective inhibitory environment in the control of animal diseases resulted in the development of immunology. The most familiar biological antimicrobial agent is the phagocyte. It may often be difficult to distinguish between a physical and a chemical antimicrobial agent since a physical antimicrobial agent may act through a chemical antimicrobial agent. Antibodies and antitoxins represent familiar chemical antimicrobial agents. Although antibodies have not been conclusively demonstrated in plants, relatively simple compounds produced by certain resistant plant varieties have been found to be chemical antimicrobial agents (reviewed by Walker and Stahmann, 1955). Chemotherapy and vaccination represent an effort to create an effective inhibitory environment in a host presenting an adequate nutritional environment but an ineffective inhibitory environment, *i.e.*, a susceptible host. Temperature may be considered to be a physical antimicrobial agent.

To demonstrate an adequate nutritional environment and an effective inhibitory environment in a host may require special techniques adapted to the specific host and parasite. If a host species includes susceptible and resistant strains or varieties, it may be possible to correlate resistance with a specific inhibitory mechanism present in the resistant strains and absent in the susceptible strains. Although it would be desirable to isolate the mechanism for an *in vitro* demonstration, such a test may not always be possible. For example, Gothoskar *et al.* (1955) correlated resistance to Fusarium in the Jefferson variety of the tomato with the production of an antimicrobial agent; it was not possible to extract the presumed responsible chemical(s). However, the high resistance of onion varieties with pigmented bulb scales to the onion smudge fungus has been correlated with the presence of protocatechuic acid and catechol (and not found in onion varieties with non-pigmented bulb scales) which act as chemical antimicrobial agents. This fascinating situation is reviewed in some detail by Walker and Stahmann (1955). Rich (1923) observed a similar situation in guinea pigs. A strain was found to be "free" of complement compared with other guinea pigs which had the normal high complement titer. The difference was controlled by a single gene, complement-less being recessive. Guinea pigs lacking complement were highly susceptible to *P. suiseptica* and *S. cholerae suis* when infected either experimentally or spontaneously. Morality in complement-less guinea pigs was 77 per cent; mortality in normal guinea pigs was 20 per cent.

THE PARASITE POPULATION

While the nutrition-inhibition hypothesis of pathogenicity emphasizes the host environment, it also assumes that the parasite remains unaltered in its characteristics. Such an assumption is valid only if it is supported by experimental evidence. Populations of parasites are relatively enormous so that mutants readily occur. Should an avirulent strain be able to proliferate to some degree in the host, it may be possible for a virulent mutant to arise and eventually to produce a population of virulent cells. In such an event, an avirulent strain may seem to have become virulent or a resistant host may seem to have become susceptible. Nutritionally competent mutants from nutritionally deficient strains would be able to proliferate or to metabolize in nutritional environments unsuited for the parental strain, *e.g.*, avirulent purine-requiring *K. pneumoniae* yielding virulent, purine independent mutants (Table 1). Mutants resistant to antimicrobial agents formerly effective for the parental strain would be virulent for formerly "resistant" hosts. For example, Garber *et al.* (1953) observed that *P. pestis* was extremely sensitive to streptomycin both *in vitro* and *in vivo*. Streptomycin therapy effective for mice infected with the parental sensitive, virulent strain was ineffective for mice infected with the mutant, streptomycin resistant strain. In this example, an ineffective inhibitory environment in the mouse for *P. pestis* was transformed into an effective inhibitory environment by the introduction of streptomycin. Consequently, the host could then be considered to be resistant to this pathogen. The occurrence of streptomycin resistant mutants of the pathogen would nullify the inhibitory effect of streptomycin. The role of mutation and selection in altering and maintaining populations of pathogens both *in vitro* and *in vivo* has been presented in considerable detail by Braun (1953).

The antigenic, morphological, and enzymatic characteristics of parasites are genetically controlled and not, at least directly, influenced by the host environment. For example, the ability to produce an enzyme is a genetic property of the parasite but the host may have to provide the necessary substrate. These characteristics of the parasite undoubtedly play an important role in the virulence of the parasite. While certain antigenic and morphological characteristics may represent a defense against certain inhibitory mechanisms of the host, other antigenic and morphological characteristics may not be significant. It may be important to establish criteria distinguishing these possibilities.

Burrows (1955) has presented an illuminating analysis of the basis of virulence for mice of *P. pestis* with relation to our current knowledge of the antigenic and morphological characteristics of this pathogen. "(phagocytosis) Resistant virulent organisms may have no observable capsule, but heavily capsulated virulent organisms are also phagocytosis-resistant, and very much more so than are avirulent cells capsulated to the same degree. It is probable that capsulated virulent organisms are resistant for the same reasons that non-capsulated virulent organisms are resistant—a situation which is understandable by assuming the presence of an invisible, but nevertheless effective, degree of capsulation in the latter. If this is so it must be presumed that the capsular material of virulent organisms has, *in its native state*, some different properties from that of avirulent organisms, despite the fact that extracted products from virulent and avirulent organisms apparently have identical properties. It is not unprecedented in immunology for biological materials to differ in physiological activity although serologically indistinguishable." (Burrows, 1955)

The probable role of enzymes has been succinctly presented by Dubos (1954). "All the microbial enzymes that we have just briefly considered take a direct or indirect part in pathological processes, but—except for the lecithinases of clostridia—their effects are difficult to evaluate because they do not cause obvious toxic reactions. It is important to notice that they do not kill the tissue cells which they attack and that some of them (for example, hyaluronidase, collagenase, dornase, fibrinolysin) appear to hydrolyze only substrate present in the extracellular environment. As a consequence, their toxic effects are only those of the split products of their specific substrates and are not apparent unless large amounts of enzymes are involved. It is probable that they affect the course of infection only indirectly by changing the physical and chemical characteristics of the environment surrounding the parasite."

It may be profitable to consider pathogenicity as a consequence of a series of events. First, the parasite must proliferate from the relatively small population in the "normal" inoculum to a characteristically large population. Second, the parasite must be able to produce the necessary materials (enzymes?) to alter the immediate environment in the host if such an alteration is required. Third, the parasite must be able to affect the host *in toto* or specific cells or tissues of the host by producing specific materials (enzymes, toxins, antimetabolites, *etc*.). Failure to accomplish any one of these steps may result in avirulence. A study of "the biochemical determinants of microbial diseases" (Dubos, 1954) may yield more far-reaching information than has yet become available from current techniques.

SUMMARY

A nutrition-inhibition hypothesis of pathogenicity is proposed on the basis of an ecological point of view. This hypothesis considers the two environments in the host which directly affect the fate of an invading parasite: a nutritional environment and an inhibitory environment. The nutritional environment may be adequate or inadequate to support a proliferating or metabolizing population of the parasite; the inhibitory environment may be effective or ineffective in suppressing the proliferation or metabolism of a population of the parasite. Of the four possible combinations, only the adequate nutrition-ineffective inhibitory environment results in virulence. Examples of virulence and avirulence as a consequence of the parasite encountering specific host environments are presented. The possible role of the antigenic, morphological, and enzymatic characteristics of the parasite as they relate to pathogenicity is discussed.

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LETTERS TO THE EDITORS

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MODES OF TETRAD FORMATION

A fundamental concept in the theory of genetic linkage is that of the mode of formation of the meiotic products, whether these be tetrads or single gametes. In the study of the gametic output of diploids this idea is a fairly familiar one. A diploid organism heterozygous at two linked loci may produce gametes of four different genotypes but there are only two different modes of formation, corresponding with the presence or absence of recombination between the two loci. In general, a diploid organism which is heterozygous at any number n of linked loci may produce gametes of 2^n different genotypes but there are only 2^{n-1} different modes of gamete formation, corresponding with the presence or absence of recombination in each of the $n - 1$ intervals. Any mode of formation can lead to gametes of two different genotypes which are determined by the parental genotype.

With tetrads, there are not only many more genotypes and modes of formation than there are for the gametic output but the ratio of the number of tetrad genotypes to the number of modes of formation depends on the number of loci considered. With a given number of loci, some modes of formation lead to one and others to two tetrad genotypes.

Ordered Tetrads. In an ordered tetrad, the two pairs of products of the first meiotic division can be distinguished and this distinction must be shown in the genotype of the tetrad. If W, X, Y and Z represent the four products of a single meiosis in which W and X separated from Y and Z at the first division, the genotype of this ordered tetrad may be written in the form

W, X ; Y, Z.

No significance is to be attached to the order of arrangement within either pair or between the two pairs of products and the given genotype therefore could also have been written in any of the following equivalent forms:

Y, Z ; W, X
W, X ; Z, Y Z, Y ; W, X
X, W ; Y, Z Y, Z ; X, W
X, W ; Z, Y Z, Y ; X, W

With respect to a single gene difference A-a, there are two possible genotypes for an ordered tetrad:

A, A ; a, a and A, a ; A, a.

A diploid parent Aa produces tetrads of these two genotypes by two different modes of formation corresponding to reductional and equational separation respectively. The relative frequencies of occurrence of these two modes of formation are characteristic of the particular locus in question and must be determined experimentally.

A diploid organism heterozygous at two linked loci may give rise to ordered tetrads of seven different genotypes. If B and b denote the genes available at the second locus, these genotypes are as follows:

AB, AB ; ab, ab
Ab, Ab ; aB, aB
AB, aB ; Ab, ab
AB, Ab ; aB, ab
AB, ab ; AB, ab
AB, ab ; Ab, aB
Ab, aB ; Ab, aB .

Since every one of these genotypes is unaltered by an interchange of parental chromosomes (whether these be AB and ab, or Ab and aB), each one may be said to correspond with a different mode of tetrad formation. The specification of the output of tetrads from a parent heterozygous at two linked loci requires knowledge of the frequencies of occurrence of these seven modes of formation. These frequencies, which are characteristic of the pair of loci concerned, are not given by theory but must be determined experimentally from tetrad analysis.

With three or more linked loci, there are more tetrad genotypes than modes of tetrad formation. Some pairs of genotypes correspond with the same mode of formation. Whitehouse (1942) has demonstrated that there are 32

TABLE 1

THE 32 GENOTYPES OF ORDERED TETRADS WITH SEGREGATION AT THREE LINKED LOCI. (AFTER WHITEHOUSE, 1942)

i) AA aa

<table>
<tr><td>1</td><td>C</td><td>c</td><td>C</td><td>c</td><td>C</td><td>c</td></tr>
<tr><td>2</td><td>C</td><td>c</td><td>c</td><td>C</td><td>c</td><td>C</td></tr>
<tr><td>3</td><td>c</td><td>C</td><td>C</td><td>c</td><td>c</td><td>C</td></tr>
<tr><td>1 2 3 4</td><td>c</td><td>C</td><td>c</td><td>C</td><td>C</td><td>c</td></tr>
<tr><td>B B b b</td><td>1</td><td>2</td><td colspan="4">5</td></tr>
<tr><td>b b B B</td><td>3</td><td>4</td><td colspan="4">6</td></tr>
<tr><td>B b B b
b B b B</td><td rowspan="2">7</td><td rowspan="2">8</td><td>9
10</td><td>10
9</td><td>11
12</td><td>12
11</td></tr>
<tr><td>B b b B
b B B b</td><td>11
12</td><td>12
11</td><td>9
10</td><td>10
9</td></tr>
</table>

ii) Aa Aa

<table>
<tr><td>1</td><td>C</td><td>c</td><td>C</td><td>c</td><td>C</td><td>c</td></tr>
<tr><td>2</td><td>C</td><td>c</td><td>c</td><td>C</td><td>c</td><td>C</td></tr>
<tr><td>3</td><td>c</td><td>C</td><td>C</td><td>c</td><td>c</td><td>C</td></tr>
<tr><td>1 2 3 4</td><td>c</td><td>C</td><td>c</td><td>C</td><td>C</td><td>c</td></tr>
<tr><td>B B b b
b b B B</td><td>13
14</td><td>14
13</td><td>15</td><td>16</td><td>17
18</td><td>18
17</td></tr>
<tr><td>B b B b</td><td colspan="2">19</td><td>21</td><td>22</td><td colspan="2">25</td></tr>
<tr><td>b B b B</td><td colspan="2">20</td><td>23</td><td>24</td><td colspan="2">26</td></tr>
<tr><td>B b b B
b B B b</td><td>27
28</td><td>28
27</td><td>29</td><td>30</td><td>31
32</td><td>32
31</td></tr>
</table>

different "classes of arrangement" of the four products in the case of three linked loci and 172 for four loci. These are in fact the different genotypex. For three linked loci they are shown in table 1 (cf. Whitehouse *loc. cit.* Table 3). Fisher (1950) has determined the number of modes of formation of ordered tetrads with any number of linked loci and in particular, has shown that there are 29 and 136 modes for the cases of three and four linked loci respectively. With three linked loci, there are three fewer modes of formation than there are genotypes because six of the 32 tetrad genotypes can be arranged into three pairs, the two genotypes of a pair corresponding with the same mode of formation, being obtainable from one another by a simple interchange of the parental chromosomes. For example, when the parental genotype is ABC/abc, these three pairs of genotypes are

ABC, Abc ; aBc, abC and abc, aBC ; AbC, ABc,
ABC, aBc ; abC, Abc and abc, AbC ; ABc, aBC,
ABC, abC ; Abc, aBc and abc, ABc ; aBC, AbC,

i.e., those numbered 11 and 12, 17 and 18, and 27 and 28 respectively in table 1. The two tetrads of any of these three pairs will have the same expected frequency of occurrence. The frequencies with which tetrads are produced by the 29 different modes of formation will be characteristic of the particular set of three loci concerned and must be determined experimentally.

Fisher (1950) showed that the number of modes of formation of ordered tetrads with *n* linked loci is

$$(6^n + 3.4^n + 7.2^n)/16,$$

$$2, 7, 29, 136, 692, 3712, \ldots$$

The number of tetrad genotypes, which can be obtained very readily from Fisher's analysis on neglecting interchanges between homologous genes (Aa), is

$$(6^n + 5.2^n)/8,$$

$$2, 7, 32, 172, 992, 5872, \ldots$$

Hence, as the number of linked loci is increased, the number of tetrad genotypes increases relative to the number of modes of formation until almost every mode of formation is able to give rise to tetrads of two different genotypes. It should be noted that Whitehouse (1942) gave

$$\frac{3}{2}(n+1)! - 2^{n-1},$$

$$2, 7, 32, 172, 1064, 7528, \ldots$$

as the number of classes into which ordered tetrads could be grouped, while according to Papazian (1952), 'the number of classes of ordered tetrads, pooling only those classes that give no information as to crossing-over is $(5.2^n + 6^n)/8$.' Whitehouse's formula is incorrect while Papazian has enumerated, not the classes to which he refers, but tetrad genotypes.

Whitehouse (1942) and also Papazian (1951) have distinguished two kinds of three-strand double crossovers. This is equivalent genetically to distinguishing between the two tetrads of a pair produced by the same mode of formation, a distinction that is not necessary for the genetic analysis of linkage in tetrad material.

Unordered Tetrads. In an unordered tetrad, the products of the first meiotic division cannot be distinguished. Hence the genotype of an unordered tetrad in which the four meiotic products are W, X, Y and Z, may be written in the form W, X, Y, Z where no attention is paid to the order of arrangement. An unordered tetrad which has been produced by the diploid heterozygote Aa must have the genotype A, A, a, a. With two linked loci, there are three possible genotypes:

AB, AB, ab, ab,
Ab, Ab, aB, aB,
and AB, Ab, aB, ab.

Since each of these is unaltered by interchange of the parental chromosomes, there must be three modes of tetrad formation in this case. If the parental genotype is AB/ab, the tetrad genotypes given above may be described as parental ditype (P), recombinant ditype (R) and tetratype (T) respectively. These descriptions specify the mode of formation.

With three linked loci, there are twelve possible genotypes for an unordered tetrad but only eleven modes of formation. The twelve genotypes are in

TABLE 2

THE 12 GENOTYPES OF UNORDERED TETRADS WITH SEGREGATION AT THREE LINKED LOCI, CLASSIFIED ACCORDING TO THE 11 MODES OF FORMATION BY DISTINGUISHING THE THREE PAIRS OF LOCI AS PARENTAL DITYPE (P), RECOMBINANT DITYPE (R) OR TETRATYPE (T). THE ORDER IN WHICH THE THREE PAIRS OF LOCI ARE DISTINGUISHED IS A-B, B-C, A-C. (COMPARE WITH TABLE 1(i))

AAaa

				P	N	T			
			1	C	c	C	c	C	c
			2	C	c	c	C	c	C
			3	c	C	C	c	c	C
1	2	3	4	c	C	c	C	C	c
B	B	b	b	PPP	PNN	PTT			
b	b	B	B	NNP	NPN	NTT			
B	b	B	b	TTP	TTN	TPT	TNT	TTT	
b	B	b	B			TNT	TPT		
B	b	b	B			TTT		TPT	TNT
b	B	B	b					TNT	TPT

fact those shown in table 1(i). The two genotypes numbered 11 and 12 correspond with the same mode of formation. The eleven modes of formation of unordered tetrads can be distinguished by classifying the three pairs of loci as P, R or T (see table 2).

Papazian (1952) has suggested that with unordered tetrads there is a loss of information when, instead of giving the genotypes of the tetrads, all pairs of loci are classified as P, R or T. With unordered tetrads, however, classification of all pairs of loci as P, R or T is equivalent to classification according to the mode of formation and this is all that is needed for genetic analysis.

Fisher (1950) showed that with n linked loci, the number of modes of formation of unordered tetrads is

$$(6^n + 3.4^n + 15.2^n)/48,$$

$$1, 3, 11, 48, 236, 1248, \ldots$$

The number of different possible genotypes for unordered tetrads with n linked loci is

$$(6^n + 9.2^n)/24,$$

$$1, 3, 12, 60, 336, 1968, \ldots$$

As with ordered tetrads, when the number of linked loci is increased, the number of genotypes of unordered tetrads increases relative to the number of modes of formation until almost every mode of formation can give rise to tetrads of two different genotypes.

SUMMARY

The classification of the genotypes of ordered and unordered tetrads according to their mode of formation is discussed and it is shown that as the number of linked loci is increased, almost every mode of formation is able to give rise to tetrads of two different genotypes.

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LETHALITY INDUCED BY FEEDING RADIOPHOSPHORUS TO MALE HABROBRACON

Inquires about the offspring from adult male wasps fed radioisotopes have been received both from geneticists interested in mutation and from health physicists concerned about the ecological influence of radioactive contaminants. Results available in scientific journals are either from experiments with adult females (Grosch and Sullivan, 1952, 1954a) or from larval feeding (Grosch and Sullivan, 1954b). In 1952, six months were devoted to feeding p^{32} adult male Habrobracon but this type of investigation proved to be an uneconomical expenditure of time, effort and funds. In the first place, males cannot undergo starvation as well as females (Grosch, 1950). Many become enfeebled and a few die during the necessary starvation period preliminary to feeding. Those surviving are not persistent in their mating attempts. Furthermore, even though copulations are observed, a low frequency of biparental offspring with an accompanying increase in haploid males often indicates unsatisfactory mating or lowered sperm supply even in control wasps. In general, males held the necessary lenght of time show signs of senility.

Results from the best experiment out of the many attempted are shown in table I. These are calculated on the basis of offspring per mated female. A group of 20 starved males from stock 17 were fed honey adulterated with p^{32} at a concentration of 200 uc/gm of mixture. One day after feeding, the males were mated in an outcross to virgins isolated from stock 33. In crosses of unrelated stocks, all females come from fertilized eggs, all males from unfertilized. The appearance among the offspring of an appreciable number of females, the biparental sex, indicates that the treated males were far from sterile. Additional matings by a few males still alive two days after the first mating gave similar results (not shown).

The more important aspect of the data in table I is the depression in numbers of female offspring without an accompanying increase in sons from treated fathers. As established by P. W. Whiting and associates, this provides a measure of the induction of dominant lethals. Only in a naturally parthenogenetic species can the phenomenon easily be separated from male sterility due to inactivation or failure in production of spermatozoa. In the latter cases the number of males would be increased.

For the present experimental offspring, a sex ratio of nearly 1:1 was obtained instead of the typical 60 to 70% predominance of females. When table I is compared with Heidenthal's 1945 x-ray results, the level fed is biologically equivalent to not more than 2000r. If fed to females, the same mixture can cause permanent cessation of egg production, an effect requiring 5000r of x-rays (Grosch and Sullivan, 1954a). The lowered egg hatchability caused by 2000r of x-rays can be mimicked by feeding females mixtures containing only 100 $\mu c/gm$ of p^{32}. Thus even when successful, radio-

TABLE 1

A COMPARISON WITH CONTROL DATA OF THE F 1 FROM MALES FED FROM P^{32}

One day after feeding		Eggs/ Mother	Per Cent Survival to Adults	Adults/Mother			Total ♀♀/ Total Progeny
				Female	Male	χ^2	
First Mating	T	236.91	46.15	58.09	51.24	4.25	0.53
	C	214.33	56.60	76.02	45.30		0.63
Second Mating	T	204.24	43.90	45.28	44.39	3.59	0.51
	C	233.87	55.03	77.60	51.10		0.60
Combined Total	T	441.15	45.11	103.37	95.63		0.52
	C	448.20	55.78	153.62	96.40		0.61

T = treated
C = control

phosphorus feeding experiments show considerably less effect when males rather than females are used.

In order to corroborate evidence of dominant lethality, the cocoons holding uneclosed pupae were opened and the contents identified if possible. Nearly twice as many unemerged females were obtained from treated fathers as from controls, but as expected, the number of unemerged sons found as dead pupae was identical in control and experimental fraternities. Furthermore there was a great increase in unidentifiable prepupae among offspring from p^{32}-fed males representing wasps which had died shortly after spinning. Presumably even more biparental offspring die as embryos. In one specific check of 100 offspring, 87% of those dying did so as embryos.

Studies appearing subsequent to our experiments have suggested an explanation of the relative ineffectiveness of radiophosphorus fed Habrobracon males. Autoradiographs show no radioactivity concentrated in the testis, in contrast to the female where the gonads are a main route of isotope disposal (Grosch and Sullivan, 1953). Furthermore, Pelc and Howard (1953) reported a very slow turnover for DNA phosphorus, and King (1953) emphasized that synthesis of new DNA is a foremost requirement in the mutagenic functioning of recoil and valence changes. Synthesis is not occurring in the testes of adult Habrobracon males since Henschen (1928) showed spermatogenesis to be largely completed by the time of eclosion. Therefore, heritable changes, if any, are induced by emanations from the soma and the gut contents. Finally, since in natural conditions braconid males will rather mate than eat, the ecological influence on the braconid species of ingested radioactive material should be relatively slight.

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ADAPTIVE AND SELECTIVE VALUE

We are glad that Li (1955) has entirely confirmed our conclusions (1954) as to the uselessness of w in comparing populations, and of selection coefficients of alternatives within a population for estimating the adaptiveness of populations homozygous for one alternative. Nevertheless, he asserts that we have deeply misunderstood the analytical method employed in gene frequency analysis. However, his comments clearly indicate that he has himself misunderstood our paper. He agrees that a standardisation of terms and avoidance of loose language is desirable, but overlooks the fact that it is *not* true that one word is as good as another if precisely defined; that depends on the accepted usages of the words in question. No one is going to say that we may as well call men fruitflies if the terms are defined precisely enough; and one of our points is that adaptation has had on the vast majority of occasions a useful meaning quite different from that of selection, that the use of adaptive value for selective value (or selection coefficient, or its derivatives) now leads to serious confusion, and that it is precisely this confusion that has led Dobzhansky to put forward the arguments which we have criticised, in which criticism Li himself supports us.

Li's misunderstanding is clearly seen in his treatment of the isolated sentence which he quotes from our paper and decorates with wrongly-placed italics. If italics are necessary, they should be used for the word "seems". We are commenting in this sentence that Dobzhansky, by using the word adaptive where selective is appropriate, *seems* (we do not wish to be dogmatic) to believe that populations with a high value of $\overline{w}$ are necessarily more highly adapted in the usual sense of the word than those with a low value. This comment seems justified because Dobzhansky (1951, p. 123) does use selection coefficients of alternatives within a population to draw conclusions as to homozygous populations being at a disadvantage, which only makes sense if he considers that these selection coefficients do necessarily give information on the *adaptedness* to a given environment of the alternatives taken separately. We are disagreeing with this view.

A similar misunderstanding is the basis of Li's remark that our "grasp of the intra-population selection concept is somewhat incomplete" on the grounds that no selection coefficient ever gives information on the ability to survive of homozygous populations, whereas we have said that "a particular homozygote may be at an extreme disadvantage because of extensive dislocations of its structure and physiology that result from homozygosity, irrespective of what genotypes may be coexisting with it in the same population. In this situation the selection coefficient relative to other genotypes does give a rough measure of the homozygote's chances of survival in pure cultures." The example given by Li to disprove this assertion proves nothing either way; the sort of disability we were referring to was failure to hatch from the egg at all, *irrespective of what other genotypes may be in the population.* But, as we point out immediately after the quotation given above, a mere inspection of selective values does not allow one to recognise such a situation. What is necessary is both the selective value and information on the effects of the gene or inversion in question.

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Barker, Robert G., and Herbert F. Wright, 1955. Midwest and its children. 532 p. Row, Peterson and Company, Evanston Illinois.

Beebe, William, 1955. Adventuring with Beebe. 283 p., ill. $4.50. Duell, Sloan & Pearce, New York. Little, Brown, Boston.

Selections from the writings of William Beebe, now Director Emeritus of the Department of Tropical Research, New York Zoological Society. Among the varied essays from the pen of this well-known naturalist is included an absorbing account of the first descent in the bathysphere with Otis Barton.

Berrill, N. J. 1955. Man's emerging mind. 308 p. $4.00. Dodd, Mead and Company, New York.

Future historians of the present era may well marvel at the wholesale application of scientific logic to the diversified areas of research, each of which is influenced by changing theoretical, practical and emotional interests. They will, without doubt, pay heed to the sum of high intelligence and to the eagerness of the individual scientist to produce results, and they will hardly underestimate achievements such as the unifying theories in the physical sciences, the reconnaissance of organic and ecological systems in biology, and the plans of psycho-sociologists to solve problems by concerted and prolonged analysis of human personality and culture. They finally may record earnest efforts by scientists of coming more constructively to grips with the cultural and social consequences of scientific invention.

Professor Berrill has chosen as his topic the evolution of *Homo sapiens*, against the background of organic, environmental and cultural change. He has succeeded remarkably well in reconstituting the man-nature event which now lies buried in the past, and it is not solely his simple, lucid and frequently humorous style which evokes a feeling of cosmic proportions worthy of that dramatic event. Berrill makes quite clear that man has arrived at a turning point where he must choose either to take stock of his natural resources and of his potentialities for humanized living, or perish.

This dilemma is not so obvious in the description of the early phases of human development. Here one learns, among other things, about dating rocks and bones, evolution from a small placental mammal toward ape and man-ape, early and much later "explosive" brain evolution, nakedness, tool-making, use of fire, burial rites, ancestor worship, changing art styles among stone age men, and expressions of primitive man's reverence for life. Persistence of primitive modes of behavior as well as man's most characteristic development, the "Prolongation of Youthfulness," are discussed. There are sections which deal with history and cultural change in the more modern sense, for example, with neolithic cultures, domestication, invention of the polished ax and the bow, irrigation farming, pottery wheel, bronze age civilization, the flood, Mesopotamian culture, and the still existing Hopi society. Gradually, however, the condition of man becomes more precarious on a different scale. His earlier destiny had been shaped to a large extent by the ever-changing climate and by population pressures; now the globe is filled, populations are outgrowing their food supply, and the balance of nature has changed from the many and manifold to the all-consuming one. Man's striving for adventure and freedom has remained the same nevertheless, and he may be forced to re-organize the direction in which he employs his energies. It would be wise to live within the natural budget since on this earth technological miracles have their limits and emigration to other planets remains doubtful. Man's chief difficulty now is certainly his remoteness to actual conditions and his helplessness in the face of simple creative necessities. Symptoms of man's present impotence are the dangers of atomic experimentation and extermination, the general technical hypertrophy of our time, and instances of cheap salesmanship in science. Professor Berrill is not certain that in our present society any future discovery, such as the possible control of human sex, would not be deliberately exploited to serve particular ends.

But this book has a very positive side, namely confidence in the mind and capacity of the average individual not deafened by propaganda, and it looks forward to a lofty and enlightened science that knows how to respect the laws of organic growth, for "the two most obvious characteristics of nature are loveliness and power, and when we concentrate upon power we do so at our own peril." The reader will be grateful for philosophical digressions which state the author's faith in an all-pervading unity in nature and a correspondence between the ways of nature and our own perceptive capacities. He believes that every individual life attains value and meaning in both past and future. "There is richness here if only we will give ourselves to being alive and cease struggling to disown our nature . . . . It is high time we listen."

ROBERT BLOCH

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Part 1. The flora of the Savannah River project area. 2. The Dicotyledons (Rosaceae, Mimosaceae, Caesalpiniaceae, and Fabaceae), by William R. Kelley and Wade T. Batson. 6. The mosses (cont.), by Wade T. Batson.
Part V. The amphibians and reptiles of the Savannah River project area. 3. Crocodilia, Sauria and Serpentes, by Harry W. Freeman.
Part VIII. Comparison sites. 1. The Congaree River swamp, by Willey E. Hoy. 2. The plants of the Congaree River swamp, by Cecile Huggins.

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THE
AMERICAN NATURALIST

Vol. XC July–August, 1956 No. 853

THE EARLY *AMERICAN NATURALIST* AS REVEALED BY LETTERS TO THE FOUNDERS[1]

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On November 22, 1866, F. W. Putnam, A. S. Packard, Jr., Alpheus Hyatt, and E. S. Morse, curators at the Essex Institute in Salem, Massachusetts, drew up their plans for the *American Naturalist.* These four young naturalists, who until a short time before had been students of Louis Agassiz, launched a publication which has continued to the present day. While it has been eminently successful in many respects, it has undergone periods, of great difficulty and uncertainty, especially in the early days of its history. L. C. Dunn (1944), the present editor of the journal, and E. G. Conklin (1944) have traced some of the highlights in the founding and early history of the *American Naturalist.* Other historical accounts will be found in the biography of E. S. Morse by Wayman (1942) and in the history of the Peabody Museum of Salem by Whitehill (1949).

At the Peabody Museum of Salem, which was organized as the Peabody Academy of Science by these same men at about the same time the journal was founded, there are twenty-six bound volumes of letters sent to the founders of the *American Naturalist* during the first few years of its existence. These have been studied by the writer, and selections have been made from this vast amount of correspondence which illustrate some of the problems the founders faced and the reaction of readers to the journal. These excerpts, together with letters possessed by the founders' families, form the basis for the present report, which portrays the early *American Naturalist* as seen by correspondents.

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[1] Grateful acknowledgment is made to Ernest Stanley Dodge, Director of the Peabody Museum, Salem, Massachusetts, for permission to study records on file in that institution and for an early photograph of the museum; to Miss Alice Putnam for the privilege of reading her father's papers deposited in the Archives of Harvard University and for the loan of a portrait of Frederic Putnam; to Mrs. Frances Packard McClellan for the loan of her father's diary and a portrait of A. S. Packard; to Mrs. Harriet Hyatt Mayor for loan of letters belonging to her father, Alpheus Hyatt; and to Dr. L. C. Dunn for the photographic reproductions of the title page and table of contents of the first issue of the *American Naturalist.*

In organizing the journal F. W. Putnam was placed in charge of the printing since he founded and owned the Salem Press which printed this pioneering magazine. A. S. Packard had charge of literary matters and did most of the editing over a long period of time. Alpheus Hyatt was in charge of the business affairs and kept the books, while E. S. Morse took charge of subscriptions and mailings. He also prepared many of the illustrations, being one of the most skillful scientific artists of his day. W. S. West was hired to help with the clerical work and Caleb Cooke, a staff member at the museum, also assisted in the routine tasks of issuing this publication.

The American Naturalist was the first illustrated popular magazine of science produced in this country. In the first three volumes all four men are listed as editors. In volumes four and five Packard and Putnam are named as editors, while Morse and Hyatt are listed as associate editors. The following four volumes record Packard and Putnam as editors with R. H. Ward as associate editor in charge of the Department of Microscopy. Beginning with volume ten the journal was published by H. O. Houghton and Company of Boston, but Packard continued as its editor. It was published continuously until 1878 under the auspices of the founders, after which time it passed into other hands. The founders, however, continued to give it support and made contributions to its pages over a long period of time.

In the first issue the young naturalists stated their purpose and aspirations as follows: "We shall endeavor to meet the wants of all lovers of nature—shall popularize the best results of scientific study and thus serve as a medium between the teacher and student. Shall it do no more than to bring naturalists, both young and old, into an active co-operation and sympathy, and promote good fellowship and amity between the great brotherhood of enthusiasts, as all true naturalists are, we shall gain a most important object.... We trust the Magazine will be equally welcome to the farmer, gardener and artisan. We shall endeavor to point out the practical benefits resulting from the study of nature."

Packard wrote enthusiastically to his father upon returning from a business trip to New York on Mar. 17, 1867, as follows: "I was on the go all day and did heaps of business for the *Naturalist* and learned much of the art of magazining, to coin a word, and of the methods of pushing the magazine with the market. I was glad to find on my return that we have already sold over 1,000 copies! Shall have to print 2,000 of number 2. It is going like hot cakes!" At the end of the second year of publication, 2,500 copies of each issue were printed at a cost of $500. The actual circulation at that time was about 2,100. Packard's father, a professor at Bowdoin College, wrote to his son on Mar. 11, 1868, "I think your last number of the Naturalist an excellent one. Your own articles always read very well."

The journal was well received by both scientists and laymen. A review in the *Quarterly Journal of Science* (London) stated that, "The leading men of science in America are among its contributors, and it is in every way worthy of the great nation which it is intended to interest and instruct."

FIGURE 1. Frederic W. Putnam, publisher of the early *American Naturalist.*

C. H. Hitchcock wrote on May 15, 1867, "My dear Packard.—I first saw in Paris a prospectus of your *Naturalist* and a copy of the first number at the house of Mrs. Marcou. I admire your courage and perseverance in attempting such an arduous work and wish you great success." Prof. Marcou, himself, later wrote that, "C'est une bonne et utile publication." Theodore Lyman wrote on June 19, 1867, "Prof. Agassiz directs me to acknowledge the receipt of numbers 1, 2, 3, 4 of your very valuable and handsome publication sent for the library of the museum and to return you his sincere thanks for the same." Among the lay readers such comments as the following were received: From Brownsville, Pa., Aug. 16, 1867, "A friend has kindly sent me some specimen numbers of your magazine. It is *just the thing* that I have been looking for these five years past. It shall not die for want of my support. I enclose $3.00 for which please send to my address the *Naturalist* for 1869." Another letter from Brooklyn, N. Y., Feb. 1, 1869, reads, "I enclose $4.00 for the *Naturalist* this coming year. The work is not only valuable to the general reader, but to the student and scholar as well—and is in every respect a credit to the Institute from which it emanates. I hope during the present year to visit the Peabody Institute. It seems to have more life and genuine enthusiasm among its workers than any other similar one in the country." Lt. Charles Bendire, U. S. Army, wrote from Fort Lapwai, Idaho Territory, on Jan. 10, 1869, "Looking through an old file of papers of the year '67, I saw a brief article on the first appearance of the *Naturalist*. I have been wanting to become a subscriber of a paper or a magazine of this sort published in the United States. Please send me a number, if possible the first issued in '69, as a sign of the Naturalist's existence and I shall remit the subscription price for the year with pleasure, by return mail."

The magazine seemed to fill admirably the need for which it was intended. A letter from Dayton, Ohio, August 21, 1867, reads, "I am much pleased with your magazine. It is just such a magazine as the student of natural history has long since wanted, and I hope you will meet with all the encouragement you need." Another one from Syracuse, N. Y., July, 1867, says, "Some such publications shoot over the heads of common people and are food only for those far advanced. The mass needs reliable plain common-sense relations and descriptions from keen-eyed observers. Let the common and scientific twins go hand in hand so that he that reads may read and understand what he reads and learn where to look and how to look and how to be benefited himself or interest others in what he may discover." For some subscribers the magazine served as an outlet for those in search of serious literature. A letter from Monticello, Iowa, Dec., 1868 gives this point of view, "The study of natural history needs to be encouraged among the people. If it could take the place of much of the trashy literature of the day it would do more to educate them and to support their wants and better satiate their mental needs." And again from Greencastle, Ohio, Feb. 19, 1869, "As I wish to make natural history my special study and not having the

FIGURE 2. A. S. Packard, Jr., principal editor of the early *American Naturalist.*

means to purchase those large and valuable works on that subject, I shall rely upon you and your valuable magazine for various instructions."

A good deal of correspondence was received concerning contributions from naturalists of the day who had apparently been solicited for papers. Samples of this type of correspondence follow. From F. V. Hayden (Director of Survey of the Territories) Omaha, Neb., Aug. 20, 1867, "I will send several articles on Indians with wood-cut illustrations—3 or 4 each.... I wish you would notice the Geological Survey of Nebraska in your journal. Success to you in your laudable efforts." Then from Colorado on Nov. 20, 1867, Hayden wrote, "My dear Packard, I will do all I can to write you some articles but I am driven almost to death."

Joseph Henry, Secretary of the Smithsonian Institution wrote on Nov. 4, 1868, "We enclose some interesting articles on the *Habits of the Burrowing Owl* and the *Shedding of the Horns of the Antelope* (the former by Dr. Canfield, the latter by Mr. Hurman) which you are at liberty to publish in the *American Naturalist* giving proper credit to the Smithsonian Institution." He wrote again on Dec. 11, 1869, "Herewith we enclose the manuscript of an article on *The Sea Otter* by Capt. Scammon of the U. S. Revenue Service in order that it may be published in the *Naturalist* if you desire it for that purpose."

A. E. Verrill wrote from New Haven, Conn., Nov. 5, 1868, "I hope to write an article soon giving a sketch of our dredging trip and noting some of our more interesting discoveries." And another one from New Haven written by O. C. Marsh dated Nov. 27, 1868, "My dear Hyatt, Your note requesting a review of Dana was duly received. As I have just returned from another western trip, I am particularly busy and so not to disappoint you, I have asked Prof. Banker to write it."

Trenton, N. J., "I send you today, my first opportunity, the article on fish of which I had made mention. I have spent much time over it culling the sweets of all 2,000 pages of notes taken down at odd times not far apart since the summer of 1855." Charles C. Abbott.

New Haven, Conn., May 10, 1869, "I am sorry to say, in reply to your instruction to write an article for the *Naturalist* on the crabs of our coast, that my time is so occupied that it will be impossible for me to do anything of the kind this term—Mr. Verrill has written to you this evening and has also begun his article for the *Naturalist*." S. I. Smith. Chicago Academy of Science, Nov. 3, 1869, "My dear Packard, I returned the proof yesterday—I can't promise about the Florida paper. My time day and evening is about entirely taken up by Academy duties for I have to do everything that requires any knowledge beyond that of an ordinary clerk—I envy you Salem fellows and often wish I could get together such a coterie of savants here." William Stimpson.

From the letters of Edward D. Cope the following are quoted. Hall of the American Museum of Science, Jan. 15, 1869, "I send a rough sketch of a plate I want cut in wood to illustrate the *American Naturalist* if you will have it. It will accompany part two of the *Fossil Reptiles of New Jersey*

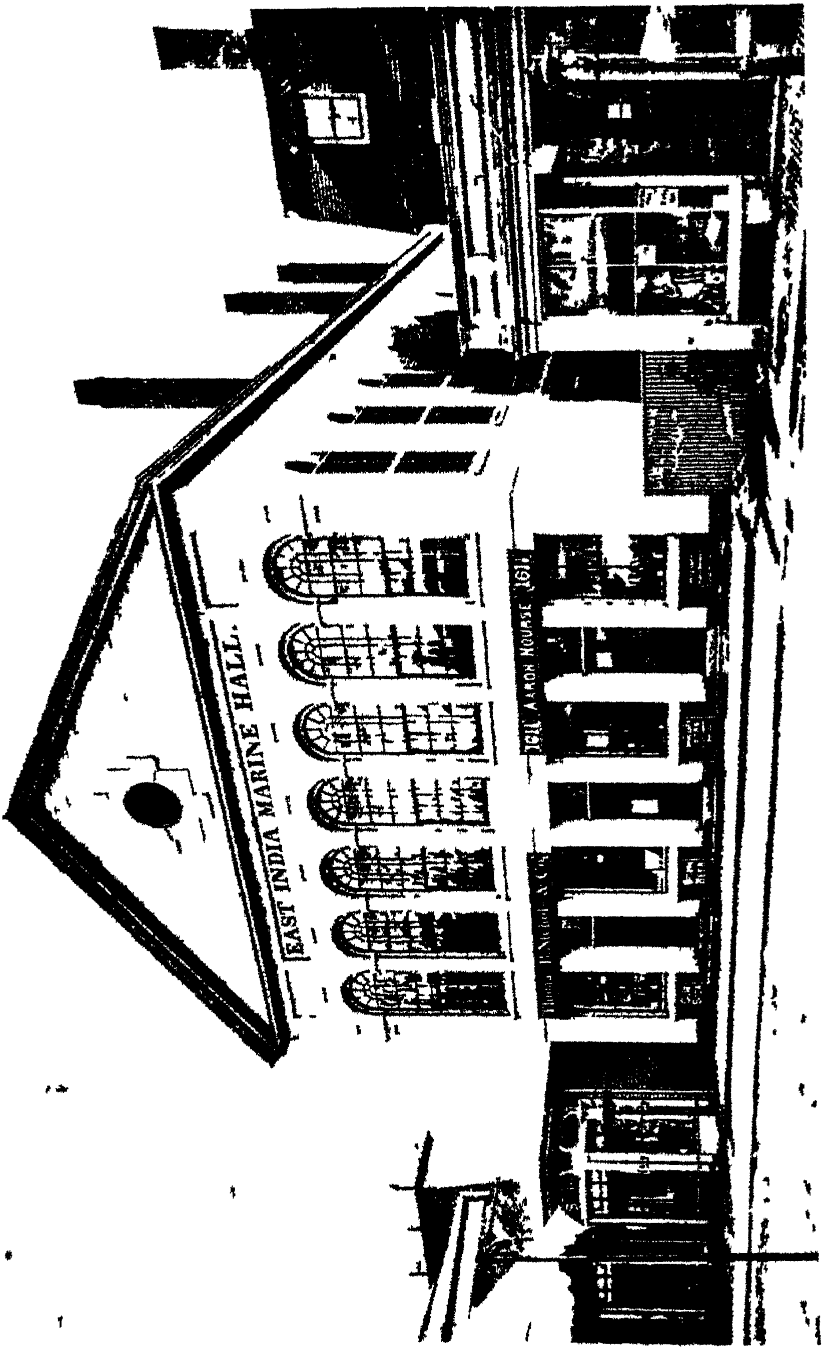


FIGURE 3. Peabody Academy of Science in Salem at which the *American Naturalist* was founded. Photo taken about 1870.

which will occupy eleven pages or so of the *American Naturalist*.... I hope that whoever draws it on wood will replace the dark lines by fine lines and shade, etc., with a true artist's eye. This any good man can do: I have not time for more than the animals and the scene. Cannot your cutter be the one who did Wood's *Phalangiidae?* The work ought to be at least as good as that done for the illustrated papers, which our scientific cuts usually are not." Philadelphia, June 13, 1869, "I hope to fulfill my promise to send you an essay on whales one of these days." Haddonfield, N. J., March 7, 1870, "I will write in a few days the desired review of Leidy's book."

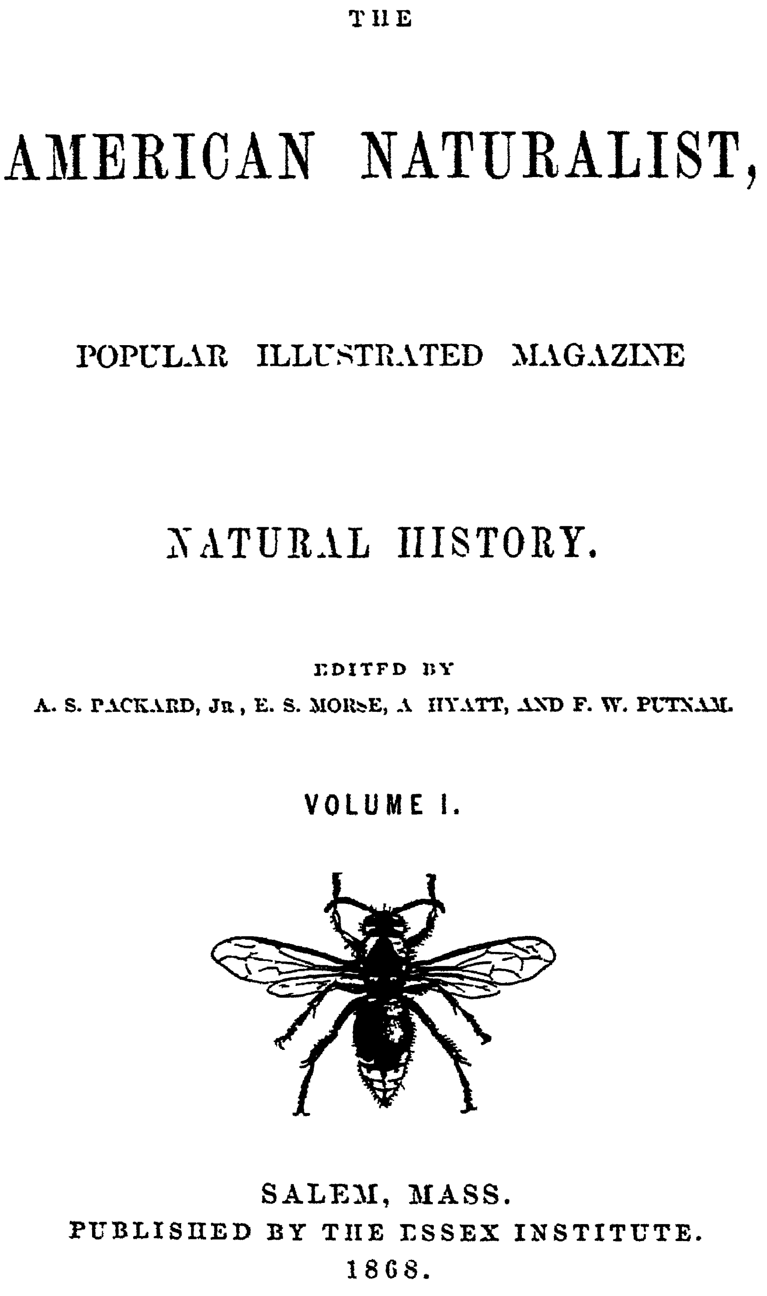
Cambridge, Mass., Jan. 19, 1869, "I presume there would be no difficulty about the wood cuts certainly not as far as I am concerned. You can have them no doubt by applying to S. J. Scudder in whose possession they are. I am rather hard pressed for the present but have no doubt to be able to send you something before many weeks for the *Naturalist*." Jeffries Wyman. Ft. Macon, N. C., March 26, 1870, "I had rather intended a sketch of all our sandpipers but found the article was getting too long and so left off at a good stopping place. If you have no more eligible matter for the July number I could work the rest into a separate article." Elliott Coues.

February 5, 1869, "I am glad that the manuscript was of use and will do the same for you again when leisure offers." William H. Dall. Later he wrote, Smithsonian Institution, April 4, 1870, "My dear Packard, Better late than never and I enclose a short article for you as I promised and if you like it, I will follow it with others by and by. Please tell Hyatt that I think the Chicago Academy will illustrate the Polyzoa, at all events he had better make drawings of them. Give my regards to Putnam, Morse, and the rest and believe me. Ever truly yours, William H. Dall."

In addition to the professional naturalists who were solicited for articles many amateur scientists sent voluntary contributions. Typical of such letters is the one from Mackerel Corner, N. H., Dec. 1869, "I send you a few items from my notebook and if these notes are acceptable will send others —I do not write from but what I know and have seen with my own eyes." From the Smithsonian Institution, Apr. 22, 1869, "I enclose an interesting article—*The Arkansas Fly Catcher*—which you may like to print in the *Naturalist*. It was written by Robert Ridgeway, one of our new ornithologists and of much promise." Spencer F. Baird.

From Fort Paul, Minn., Oct. 3, 1868, "I have a great amount of information in regard to the surface geology and some important general principles derived from them which I would be glad to furnish you with hereafter if I can get time from my official duties." G. K. Warren (U. S. Army).

Whenever there was any question as to the value of any voluntary contribution, advice was sought from those qualified in the field concerned. We find such letters as the following. Cambridge, Sept. 26, 1868, "You asked if certain notes of William P. Bolles are worth publishing. I should hesitate, though that is what half the English local literature consists of—new localities and observations upon the most trivial variations." Horace Mann. And again from Horace Mann on Oct. 4, 1868, "I beg you will not advertise



THE

AMERICAN NATURALIST,

POPULAR ILLUSTRATED MAGAZINE

NATURAL HISTORY.

EDITED BY
A. S. PACKARD, Jr., E. S. MORSE, A. HYATT, AND F. W. PUTNAM.

VOLUME I.

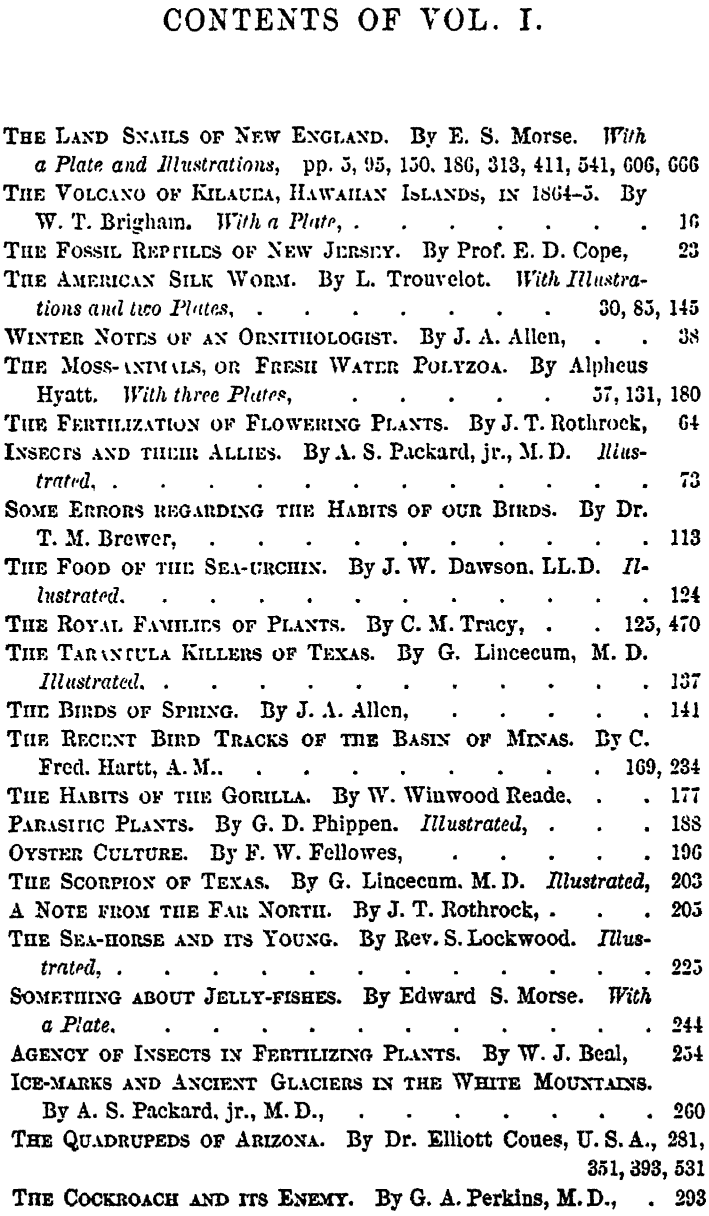
SALEM, MASS.
PUBLISHED BY THE ESSEX INSTITUTE.
1868.

FIGURE 4. Title page of the first issue of the *American Naturalist.*

or sell that catalogue of Canadian Plants by Hubbert. He is dead, to be sure, but it is one of the most miserable and inadequate things ever printed being a compilation from old botanies which do not begin to give the thing as it is correctly." A comment from J. A. Allen of Cambridge reads, "Jan. 13, 1870, Dear Putnam, The notes sent a day or two since in respect to our winter ducks are interesting and important. Professional naturalists rarely have the opportunity of becoming so well acquainted with this class of birds as sportsmen do."

A good deal of correspondence was received by the editors requesting information of all kinds pertaining to natural history. Some of these letters, with the answers, were published in the *Naturalist.* Others were answered privately. Samples of this type of correspondence follow. July 5, 1867, "Will you please inform me what to understand in the following, found on page 273 *American Naturalist* seventh line from top 'Man had a common origin.' When reading it yesterday I could not take from it a satisfactory sense, and I trust you will oblige me by gratifying my curiosity to understand what I read." Grand Isle, Vt., Oct. 8, 1867, "Enclosed you will find some specimens of land snails which I found in this vicinity. I hope to learn their names and send them to you for that purpose. Will you give them in your next issue of the *Naturalist.*" Sept. 18, 1867, "I cannot find any definition of *slide*. Even in Webster's large dictionary, such as I presume is meant. Since giving my attention to readings in natural history I have met with many words not to be found in the more common dictionaries." Nov. 8, 1868, "Can't you give me a recipe in the next *Naturalist* for a mucilage or glue with which to affix numbers and other labels to minerals and which will resist both damage and dry atmosphere?" Nov. 24, 1868, "Can you give me information in relation to the so-called *monster* on exhibit in Boston at corner of Union and Hanover streets? Is it a humbug like Barnum's mermaid or a monstrosity or a distinct species of shark or its allies?—I questioned the exhibitor in relation to the teeth, bones, food, etc., of the beast but found his accounts somewhat conflicting."

The intention on the part of the founders to have the *Naturalist* serve as a source of information on economic entomology seems to have met a real need during the early years of publication. Some of the correspondence concerning these matters is extracted below. April 15, 1867, "Every farmer in Kansas would be greatly profited this season to understand the natural history of the red-legged locust which covered the country last August and September destroying all the land crops.... I consider the *Naturalist* a necessity to the farmer, especially the Kansas farmer." Baltimore, May 20, 1867, "Will you not permit me to suggest that you publish in your excellent journal common sense articles on *Cimex lectularius* (bedbug) and *Musca domestica* (housefly)." Tehama, Calif., May 30, 1867, "I particularly desire to see the articles on the American silkworm as silk culture is attracting much attention in California. Our soil and climate appear to be significantly adapted to that enterprise. From present appearances it is not impossible that in twenty years silk growing will be the leading interest of California."



CONTENTS OF VOL. I.

The Land Snails of New England. By E. S. Morse. *With a Plate and Illustrations*, pp. 5, 95, 150, 186, 313, 411, 541, 606, 666
The Volcano of Kilauea, Hawaiian Islands, in 1864–5. By W. T. Brigham. *With a Plate*, ... 16
The Fossil Reptiles of New Jersey. By Prof. E. D. Cope, 23
The American Silk Worm. By L. Trouvelot. *With Illustrations and two Plates*, ... 30, 85, 145
Winter Notes of an Ornithologist. By J. A. Allen, . . 38
The Moss-animals, or Fresh Water Polyzoa. By Alpheus Hyatt. *With three Plates*, ... 57, 131, 180
The Fertilization of Flowering Plants. By J. T. Rothrock, 64
Insects and their Allies. By A. S. Packard, jr., M. D. *Illustrated*, ... 73
Some Errors regarding the Habits of our Birds. By Dr. T. M. Brewer, ... 113
The Food of the Sea-urchin. By J. W. Dawson, LL.D. *Illustrated*. 124
The Royal Families of Plants. By C. M. Tracy, . . 125, 470
The Tarantula Killers of Texas. By G. Lincecum, M. D. *Illustrated*. 137
The Birds of Spring. By J. A. Allen, ... 141
The Recent Bird Tracks of the Basin of Minas. By C. Fred. Hartt, A. M.. . . . . 169, 234
The Habits of the Gorilla. By W. Winwood Reade, . . 177
Parasitic Plants. By G. D. Phippen. *Illustrated*, . . . 188
Oyster Culture. By F. W. Fellowes, ... 196
The Scorpion of Texas. By G. Lincecum, M. D. *Illustrated*, 203
A Note from the Far North. By J. T. Rothrock, . . . 205
The Sea-horse and its Young. By Rev. S. Lockwood. *Illustrated*, ... 225
Something about Jelly-fishes. By Edward S. Morse. *With a Plate*. 244
Agency of Insects in Fertilizing Plants. By W. J. Beal, 254
Ice-marks and Ancient Glaciers in the White Mountains. By A. S. Packard, jr., M. D., ... 260
The Quadrupeds of Arizona. By Dr. Elliott Coues, U. S. A., 281, 351, 393, 531
The Cockroach and its Enemy. By G. A. Perkins, M. D., . 293

FIGURE 5. Partial Table of Contents of the first volume of the *American Naturalist*.

Rockford, Ill., July 26, 1867, "If you haven't already, I hope you will give us something on the potato bug—which is committing great ravages entirely destroying whole patches of potatoes."

The early magazine was also intended to stimulate youth in the study of natural history. A letter from the mother of Bryant Walker, who later became a leader in American Conchology, reads: "Detroit, Mich., Sept. 11, 1867, Enclosed you will find $3 for the present year's issue of your magazine of which I have seen a number which has highly interested me and delighted my son Bryant Walker, a lad of eleven years of age who is enamored of the study of natural history and is already conversant with Audubon and Wilson's ornithology. He is taking lessons in taxidermy, etc." A letter from Kennebunk, Maine, Nov. 11, 1867, "Mr. Morse, I have been reading the *American Naturalist* and was very pleased by the descriptions given by you of the land snails of New England, and I am going to try to get a collection—I am only thirteen years old but I like the study of natural history better than anything else." Oberlin, Ohio, Feb. 24, 1868, "I have only been taking your magazine but one year and I am well pleased with it. I am but a boy and very fond of the study of nature, and you may look for my subscription at the close of each volume." One lad wrote, "I am very much interested in the study of nature and expect to become a naturalist. If you who are specialized would write an article entitled 'Letter to a Young Naturalist' it would, I think, just suit many who read your magazine." Another wrote from Winchendon, Mass., Aug. 19, 1869, "Enclosed is a manuscript which, if it possesses sufficient merit, I should like to see in the columns of the *American Naturalist*. In case it should meet with your approval, I will become an occasional contributor to your timely, valuable magazine. As an apology for the mistakes which you may possibly detect in the accompanying manuscript, I would state that I am but sixteen years old, and although I have written for the press, I have never before written an article for a strictly scientific journal. Will you please write me your honest opinion of the article and oblige." W. J. Beal wrote from Chicago, Ill., Dec. 27, 1869, "I am doing what I can in and about Chicago to raise up a race of naturalists."

From the very beginning the *Naturalist* had many financial difficulties. John Baldwin Coolidge wrote from Lawrence, Sept., 1867, "I am very sorry to learn that the magazine is not paying its way.... I will do all in my power to help you." Among the reasons for the financial problems which arose were the numerous requests for free subscriptions and special discounts. Unfortunately a great many people who were most interested in the magazine found the cost of the subscription to be a burden if it could be afforded at all. Among those making a request for free copies or reduced subscription rates we find the following. July 1, 1867, "Do you make any discount for clergymen and teachers?" March 12, 1869, "As president of the Reading Public Library I wish to know if you make any different terms with librarians from the advertised ones. The town of Reading is poor in money but exceedingly rich in wants." Oberlin, Ohio, Sept. 18, 1869, "As treasurer of the Reading Room Association of this place, I write to you to ascertain the price per year of the *Naturalist.* We have been able thus far to secure some deduction from the regular prices of the different periodicals. I wish you might send me a specimen copy for the committee to examine and state the lowest possible terms to which it can be had." East Boston, Mass., Jan. 11, 1870, "Is it asking too much to request you to donate your publication to our association (Y.M.C.A.)?" Sept. 3, 1869, "I am anxious to become a subscriber to the *American Naturalist* but I am unable to do so for want of funds. Can I pay for it from volume one onward in any objects of natural history in the region which you may desire?" A correspondent from Ohio sums up the situation which has been unfortunately too often true: Oct. 7, 1867, "Everywhere I find a taste for light reading and so little interest taken in anything of a scientific nature that the people seem quite unwilling to pay for it unless they can get it cheap."

Other than financial, the main problems were concerned with improper mailing and billing of the magazine. Many of these were undoubtedly attributable to the fact that there were too many persons concerned with the publishing of the magazine. Often times, one of the four was not aware of what some of the others were doing which lead to frequent confusion. Also there were occasional clerical errors of mailing and keeping records which gave rise to complaints on the part of subscribers. The following examples will illustrate some of these problems. Waddington, N. Y., Aug. 7, 1867, "I have written several letters about my July number of the *Naturalist* being missing but you do not seem to understand me for I am still getting August numbers—have three now. I should like a July number very much to fill my set." Otter Creek, Ill., July 6, 1867, "A short time ago I sent $3 for a years' subscription to the *Naturalist* and yesterday a package of four came to hand containing a receipt—when lo!! and behold!!! they were all alike —the March number—which was most too much of a good thing seeing I had had a copy sometime ago and read it several times." San Francisco. Sept., 1867, "Unless packages are sent by steamer they will not be likely to reach this office. Large quantities of mail matter lie on the overland route and is used to fill up holes beside cannons." Exeter, N. Y., May 10, 1869, "Where is my Naturalist? I hear that my article is out and have been wanting to see how it looks so as to judge about writing another. J. W. Chickering, Jr." New York, Sept. 19, 1867, "There appears to be a 'screw loose' in your *American Naturalist* affairs. I have just received a third bill for the *Naturalist* for 1867 with the request that I remit 'early' the sum of $3. I find I have a receipted bill for this signed 'A. Hyatt' and dated April 3, 1867." Chicago, Ill., Sept. 23, 1867, "For the third time I am favored with your bill for this year's magazine. Twice have I stated that I have paid." Academy of Natural Science, Philadlephia, Dec. 21, 1869, "I returned to you today the last number of the *Naturalist* addressed by you to Dr. Leidy, it being imperfect. Please send another number to the Academy as soon as possible."

Complaints from authors were usually concerned with editorial changes or alterations which displeased the original writer. We find, for example, letters such as the following. "Newtonville, Mass., Sept., 1869, I regret to find that although I expressed my regrets in the proof of my article on the 'Supposed New Columbine and New Ox-eye' that the names of Miss Grace H. Crockett and Mrs. Alida C. Avery had been omitted as the discoverers of the daisy. The article appears without the names. This omission may seriously compromise me here among the students and officers and I regret exceedingly that you should find it necessary to depart from the ms. for there is a pride felt about the discovery." North Madison, Conn., Feb. 24, 1869, "You did not consider the notes on the breeding habits of a few of our birds which I sent you some months since worth publishing. I may be egotistical in thinking them as interesting as some which have since been published." W. Newton. Mass., Aug. 12, 1869, "I have just read in the August number of your magazine the communication I sent you on the Tennessee Warbler and am displeased at two changes you have taken the liberty to make (unless I am very much mistaken) from the original."

Much of the correspondence from authors concerned the preparation of illustrations. Two typical ones are given here. Office of the State Entomologist, St. Louis, Dec. 29, 1868, "Friend Packard, I don't know when your plate on which you figure the moth I sent will be cut but my lithographic figure will be in a short time and will perhaps have the priority. Therefore, if I adopt your name I want *your own* description.... I would like you to send me the complete technical description which you have drawn up for your own use." C. V. Riley. From New Haven, Conn., March 4, 1870, "My dear Packard, I find on examination that I have not got a decent proof of the head of Branchipus although the negative is good. So in regard to the Artemiae and Branchinecta—the proof which I enclose is by no means as good as we can get—We had better wait a few days until Smith (Sidney I. Smith) can get a chance to make some more proofs. Perhaps it would be well to engrave one of the heads of Branchinecta or perhaps its egg case in order to make the paper more complete." A. E. Verrill.

Many requests were received for publicity. Some of these were commercial and others of professional interest. Manufacturers of scientific apparatus wrote such offers as "I will send you a microscope (the Craig microscope) prepaid with some mounted microscopic objects if you will notice it editorially in the *American Naturalist*." Professional notices were requested such as the one from C. H. Hitchcock from Hanover, N. H., Feb. 25, 1869, "If you choose to note briefly the fact of an organization of a Geological Survey of New Hampshire, I shall not object." Offers for exchange notices were numerous. One from the *Maryland Educational Journal* read, "May 20, 1867. I am willing to give you good notices in return for the same on your part towards this journal in as much as we too (to quote your words) also 'need every encouragement in starting our enterprise'." From the University of Michigan, Ann Arbor, Jan. 20, 1869, "We have sent you a copy of our University Magazine hoping to be able to place your publication on our exchange list. We do not expect you will receive an equivalent for your publication in our magazine, but we give extended notices of our best exchanges."

Zoology has always been the main interest of the *Naturalist* although in the early years there was a section on botany and one on microscopy. A letter from Asa Gray suggested that the *Naturalist* increase its service to botany. It read in part, "Botanic Gardens, Cambridge, Mass., Feb. 14, 1870, I am telling our scattered botanists they should make the *Naturalist* their organ of communication, and I think it would be to your good to enlist botanists more. There is a plan in Illinois to extend the *American Entomologist* to include botany. I tell them better support one journal well, such as yours, than have more and feeble ones. If you will take to botany I will endeavor with Mr. Hughes' help to aid you."

Several letters show a prevailing interest in the work of Charles Darwin, whose *Origin of Species* had only recently been published. A surprising letter was received by Dr. Packard from C. H. Hitchcock, writing of his trip to England, dated May 15, 1867, as follows: "I also attended 80 lectures on zoology by Professor Huxley who is truly a live practical naturalist not carried away by Darwinism enough to injure the integrity of his researches. He presented both sides of the development question more fairly than it has been done by any Progressivist I have met—so much so indeed that tho' I had a leaning towards Darwinism his facts have cured me of it."

About that same time a paper on the American Deer with evolutionary bearing was published by J. D. Caton in the *Transactions of Ottawa Academy of Natural Science*. This stimulated a good deal of comment in the pages of the *American Naturalist*. The author sent the following note to the editor with a copy of his letter from Darwin. Ottawa, Ill., Dec. 11, 1868. "I take the liberty of enclosing a copy of a letter I have received from Mr. Darwin on my paper on the 'American Cervus'.—Sept. 18, 1868, Dear Sir: I beg leave to thank you very sincerely for your kindness in sending me through Mr. Walsh your admirable paper on the American Deer. It is quite full of most interesting observations, stated with the greatest clearness. I have seldom read a paper with more interest—for it abounds with facts of direct use for my work. Many of them consist of little points which hardly anyone besides yourself have observed or perceived the importance of recording. I would instance the age at which the horns are developed (a point on which I have lately been in vain searching for information) the development of horn on the female Elk and especially to different natures of the plants consumed by the deer and elk and several other points. With cordial thanks for the pleasure and instruction which you have afforded me and with high respect for your powers of observation. I beg leave to remain, Dear Sir. Yours faithfully and obliged, Charles Darwin."

A relevant note received by the Naturalist from Asa Gray reads, Dec. 21, 1869, "The Spike-horn Deer and some other things in December number of your journal will interest Mr. Darwin. Could you mail to him the sheet of the Miscellany—to Down Beckenham, Kent? I oblige. Yours, Asa Gray."

While the journal was notably successful in accomplishing its stated aims and was conducted on a high professional level, it suffered from financial difficulties from the very beginning. In May of 1875, Putnam approached the trustees of the Essex Institute to shoulder the burden of the *Naturalist.* Receiving no encouragement from them, Putnam then approached Henry Holt to take it over. Holt wrote on June 30, 1875, that "The only safe general principle in these times appears to be to buy nothing, but my respect for the periodical would lead me to at least carefully consider any information you may see fit to give regarding its business condition and the requirements of its owners." A detailed answer sent by Putnam on July 5, 1875, summarizing the historical development and financial plight of the *Naturalist*, reads, "In answer to your note of June 30 about the *American Naturalist* I hardly know what to say except to give you a general statement as to how we have managed the magazine and the success we have had. The magazine was first started under the auspices of the Essex Institute by four of us who were unpaid officers of the Institute and with the simple and pure idea of doing missionary work in science. We did not look to making money out of the book but we did hope that in time we should make the magazine pay its expenses and enable us to use it for the benefit of our society. After about one year from the start Mr. Peabody gave the money to found the Peabody Academy of Science in Salem and we four editors of the magazine were made officers of the new Academy and put on salary. We, therefore, turned out the Magazine in the name of the Academy and made great use of it for the benefit of the Academy in many ways especially in sending it all over the world in exchange for the publications of other societies, journals, etc. In this way we had a large circulation, but as we never made special business of obtaining subscribers our subscription list was never one-quarter what it could have been made had we devoted time to it. All we could do with the scientific work that was on our hands was to edit the journal and let the subscribers come as they would, a poor way so far as money was concerned but a splendid thing for the reputation of the journal and to the credit of our Academy—then came these hard times when every business required to be well looked after and we find that we are out of pocket on the Magazine and our Trustees think that it will be outside of the duties of their trust to take the responsibilities of a business operation on their hands when their trust is for a specific purpose—we are forced to sell the Magazine for what we can get in order to have it continue in its good work which is of great object—if my letter is not a business one, inasmuch as it gives both sides of the story at once, you will the better be able to judge of the prospect of making the magazine a paying thing when taken up and managed on true business principles.—Now as to making the rate and the price. Parties who have looked into the matter have stated that we ought to get at least 10 or $12,000 for all the property and good will and I feel that it is well worth that. in fact in 1873 we were offered 10,000 for it by a respectable house that has since taken up other matters. In these times of course we do not expect so large a sum and we are open to bids below those figures.—Should you decide to purchase and take it off our hands at once, Dr. Packard and myself would agree to do the editorial work free for the rest of the year or until you make other arrangements and, if desired, Dr. Packard would take the position of editor at fair rates. My other duties have so much increased of late since my appointment in Cambridge that I should have to give up the editorial work with the Dec. number."

Twenty-two days later Holt wrote to Putnam, "I may as well tell you that I do really want to do this thing. 'lhe question is whether I can afford to." Five months later Holt wrote again, "In ordinary times I should be very glad to expand in the direction of science even so far as the commercially limited field of technicalities. But, this is no time to expand anywhere." Eventually H. O. Houghton and Company took over the publication of the journal. Dr. Packard received the following letter dated Oct. 26, 1875: "Referring to the conversation between you and our Mr. Houghton today we would say that we are willing to publish the *American Naturalist* for two years provided the gentlemen who are interested in its continuance will guarantee us against loss."

Thus, the *American Naturalist* left Salem. Only Packard, who was to continue to serve as chief editor for a period of ten years more, but who himself left Salem for Brown University in 1879, remained with the journal. After two years Houghton and Company passed the publication on to the press of McCalla and Stavely of Philadelphia. Packard and Edward D. Cope then served as joint editors. After volume twenty, none of the original founders continued in an official capacity.

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A STUDY ON THE NATURE OF AUTONOMY IN NEOPLASTIC PLANT CELLS*

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The tumor problem is a problem of growth and more particularly a problem of the growth of altered cells by more or less random division. The tumor cell is not, however, characterized primarily by its rate of growth since certain normal cell types may grow and divide at considerably faster rates than do most tumor cells. The tumor cell need not, moreover, be limited in a capacity to differentiate and functionate. Pluripotent teratoma cells possessing malignant properties are quite capable of showing a high degree of differentiation. The one biological character that distinguishes tumor cells from normal cells is the capacity for the uncontrolled or autonomous growth of the tumor cell within an organism. Without this there would be no tumors.

Autonomy of neoplastic growth has many gradations. At the one extreme are the so-called benign tumors which commonly grow slowly and remain localized in the host. At the other extreme are the most malignant cancers which invade neighboring tissue and spread throughout an organism by metastasis. The nature of autonomy remains one of the foremost problems in cancer research.

Since the capacity for autonomous growth is potentially a property of the cell itself, it should be equally capable of expression in the cells of all higher organisms whether they are of animal or plant origin. It does not necessarily follow, however, that the cellular mechanism, in terms of specific chemical reactions, is identical in members of the two kingdoms. The fundamental similarity of cells and cellular processes is, nevertheless, commonly recognized and advances in one field of investigation may assist in clarifying problems in related fields of study.

PROCESSES IN THE GENESIS OF TUMORS

One striking aspect of tumor genesis is the multiplicity of diverse processes that are seemingly capable of accomplishing the same end result. Radiant energy, hereditary factors, irritation, carcinogenic chemicals, microorganisms, and viruses have all been shown to be more or less effective as incitors of tumors in animals and plants. With the possible exception of the hereditary constitution of the host and of certain of the viruses, these factors are concerned with the inception of tumors and do not appear to be involved in the continued abnormal growth of tumor cells once the alteration of normal to tumor cell has been consummated. In an attempt to develop a concept designed to explain the action of the plurality of these diverse agents in the genesis of tumors, it has been postulated by some that these factors act as mutagenic agents and bring about the alteration of one or more genes in the affected cells. Others have suggested that the carcinogenic factors activate latent viruses present in certain cells. Still others have indicated that the uncontrolled growth of tumor cells may result from the selection or modification of certain normal cytoplasmic constituents which compete with or replace normal constituents concerned with the regulation of growth. Whatever the merits of these hypotheses, they do not give the desired information regarding the ultimate mechanism involved in the uncontrolled growth of cells within an organism. To state simply that a mutation has occurred or that a virus is responsible for tumor cell growth is in itself quite meaningless when attempting to characterize the underlying reason for autonomous growth. It is essential to define in physiological and biochemical terms the nature of the hypothetical mutation or to determine more precisely how the presence of a new self-duplicating entity in a cell affects the metabolism of a cell if an understanding of autonomy is to be achieved. It is with a consideration of this problem at a physiological level that we shall concern ourselves at this time.

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CROWN GALL OF PLANTS

The experimental system that will be subjected to analysis is the neoplastic disease of plants known as crown gall. Crown gall is unique among diseases of bacterial origin in that the inciting bacterium is only indirectly involved in the causation of the disease. Susceptible and properly conditioned plant cells are regularly altered to tumor cells in short periods of time by means of an as yet uncharacterized tumor-inducing principle that is associated with the presence of the living bacterium. Once the alteration of normal cells to tumor cells has been fully accomplished, the role of the bacterium in the disease process is completed. The continued abnormal proliferation of the altered cells becomes completely independent of the inciting organism. When, for example, virulent bacteria are inoculated into susceptible plant species and allowed to act on the cells of the host for 72 hours before being destroyed selectively by thermal treatment, large rapidly growing tumors result (Braun, 1943, 1947a). When, on the other hand, the bacteria are killed after acting on the host cells for only 34 hours, small slowly growing tumors develop. Some of these overgrowths continue to grow very slowly, become encapsulated, and after prolonged incubation they may reach moderate size (Braun, 1951). Sterile tissue isolated from the slowly growing tumors grew very slowly in culture when compared with the rate of growth of cells isolated from rapidly growing tumors, the cells of which were acted upon by the bacteria throughout a 72-hour period. Since the characteristic growth levels of these two types of tumor cells are retained indefinitely in culture, it would appear that the degree of cellular alteration attained in 34 hours is far less than that found when bacteria act on the host cells for a 72-hour period.

Slowly growing benign tumors can also be obtained by inoculating susceptible plant species with strains of the crown gall bacterium that show varying degrees of attenuation. The weakly virulent A66 strain initiates the formation of small slowly growing tumors on tomato (fig. 1,B). The highly virulent A6 strain, from which the attenuated A66 strain was derived (Hendrickson, *et al.*, 1934), regularly produces large rapidly growing tumors in that host (fig. 1,A). The T37 walnut strain is intermediate in its disease-producing ability when inoculated into sunflower and certain other hosts. Sterile sunflower tumor tissue altered by the moderately virulent T37 strain grows more slowly in culture than do comparable cells transformed by a highly virulent strain (Braun, 1953). When, instead of inoculating a plant such as the sunflower, the moderately virulent T37 strain is introduced into tobacco, the cells of which possess a high degree of pluripotency, then in place of the characteristic unorganized crown gall tumor a complex overgrowth or teratoma results. The capacity for organization of such pluripotent tumor cells is retained indefinitely in culture. A highly virulent strain of the crown gall bacterium, on the other hand, induces the formation of typical rapidly growing unorganized tumors in tobacco. In this instance the cellular factors concerned with organization are overwhelmed as a result of the alteration of normal pluripotent cells to tumor cells. There exists in crown gall, then, a variety of growth patterns. These

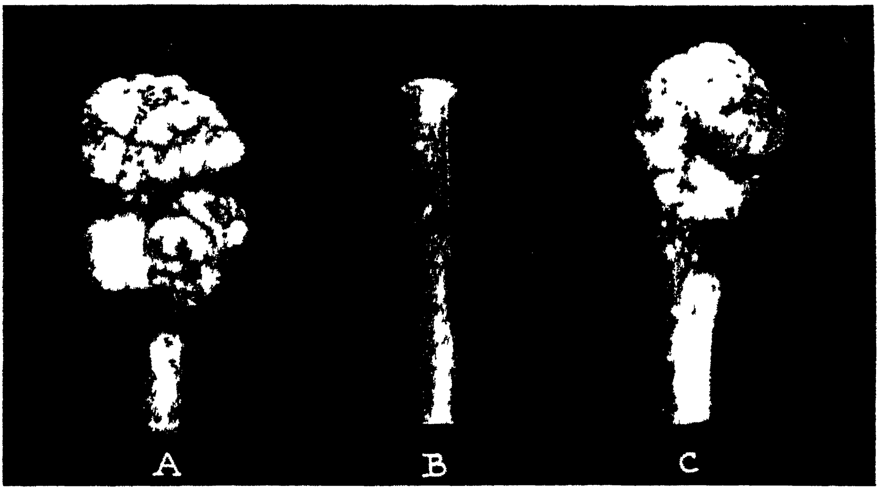

FIGURE 1. Tomato stem inoculated with: A. The highly virulent A6 strain of the crown gall bacterium. B. The attenuated A66 strain. C. The attenuated A66 culture and treated at the cut stem end with naphthalene acetic acid, a synthetic growth substance possessing auxin activity. (Photographs by J. A. Carlile)

range from slowly growing benign to rapidly growing potentially malignant tumors, and from completely unorganized to highly organized overgrowths. All have their counterparts in animal pathology. How do crown gall tumor cells achieve the capacity for autonomous growth and why do they show varying growth patterns?

GROWTH PATTERNS AND AUTONOMOUS GROWTH

Investigations carried out in a number of laboratories (Braun, 1947b; de Ropp, 1947a; Gautheret, 1947) during the past decade have suggested that uncontrolled cellular growth in crown gall is concerned with the production of greater than regulatory amounts by the tumor cell of a growth-promoting substance. Auxin synthesized by such cells had commonly been regarded as playing an etiological role in the continued abnormal development of those cells. There seems little doubt that crown gall tumor tissue is hyperauxinic (de Ropp, 1947b; Henderson and Bonner, 1952; Kulescha, 1949; Kulescha and Gautheret, 1948; Link and Eggers, 1941). However, it was by no means certain that the characteristic behavior of the crown gall tumor cell could be explained solely or even in large part on the basis of high auxin levels in those cells. Attempts were therefore made to learn whether, in addition to growth substances of the auxin type, non-auxinic factors capable of stimulating active cell proliferation are present in crown gall tumor cells (Braun and Naf, 1954; Steward, *et al.*, 1955). The method used by Braun and Naf (1954) for demonstrating such substances was similar to that used by other investigators (Steward and Caplin, 1951; Skoog, 1954) for demonstrating the presence of non-auxinic growth factors in certain normal tissues and in fluid endosperm of the coconut. Newcomb (1951) had shown that normal tobacco pith cells enlarge greatly but do not divide in the presence of growth substances of the auxin type. Cells of this tissue were therefore suitable for detecting the presence of non-auxinic growth factors capable of stimulating cell division in tumor tissue extracts. When tobacco pith tissue was isolated and planted on White's basic culture medium, the cells of the pith did not divide but remained quiescent indefinitely. When the basic medium was supplemented with naphthalene acetic acid, a synthetic growth substance possessing auxin activity, at a concentration of 1 mg/liter, the cells of the pith tissue enlarged greatly in size but they did not divide. Extracts of crown gall tissue that had been sterilized by filtration and added aseptically to the basic medium did not encourage either the enlargement or the division of the pith cells. When, however, both the auxin and filter-sterilized tumor extract were added to the basic medium in optimal concentrations, a very rapid growth by cell division of the pith cells resulted. A direct correlation existed within limits (0.005 mg – 1 mg NAA/liter) between the concentration of auxin in a culture medium containing a constant volume of tumor extract and the amount of growth by cell division that occurred in the pith tissue.

In the presence of a concentration of auxin equal to 0.5 mg NAA/liter in a culture medium containing 10 per cent tumor extract, a very active proliferation of the pith cells resulted. The growth pattern of the tobacco pith tissue in this instance showed a striking morphological and histological resemblance to tobacco crown gall tumor tissue of the unorganized type grown on the basic medium. When the concentration of auxin was reduced in the tumor-extract-containing medium to a level equal to 0.01 mg NAA/liter, the pith tissue grew slowly and in an unorganized manner for about 4 weeks. Thereafter numerous more or less organized structures covered the surface of the overgrowth. Such tissues showed a resemblance to crown gall teratomata of tobacco. It has thus been possible to reproduce under controlled conditions growth patterns that resembled, superficially at least, the morphologically distinct types of crown gall tumors that have been described as occurring on tobacco. These artificially stimulated pith tissues were, however, self-limiting and when the externally supplied stimuli were removed, their growth promptly stopped. Crown gall tumor tissue is, on the other hand, autonomous and is itself capable of synthesizing growth factors needed for its continued abnormal proliferation.

EFFECTS OF GROWTH SUBSTANCES

It seems quite clear from studies of the type reported above that at least two growth factors, one an auxin which is concerned, for purposes of this discussion, with cell enlargement and the other which is concerned with cell division, are essential if growth by cell division is to occur in tobacco pith cells. A biologically active substance possessing cell-division-factor activity has recently been characterized chemically (Miller, *et al.*, 1955). The tobacco pith cells have apparently lost, as a result of their differentiation, the capacity to produce physiologically effective concentrations of the two growth substances. Because both growth-substance-synthesizing systems appear to have been blocked in pith cells, it was believed that experiments carried out with the use of such specialized cell types might give insight into the nature of the cellular alteration in crown gall (Braun, 1956). If, for example, only the auxin system is activated as a result of the transformation of normal cells to tumor cells, then the altered pith cells should enlarge greatly without, however, dividing. If, on the other hand, the cell-division-factor synthesizing system is activated without a corresponding activation of the auxin system, then neoplastic growth should not result because, as indicated above, the cell-division factor without auxin is ineffective in initiating growth by cell division in tobacco pith cells. Only if both growth-substance systems are activated simultaneously following the alteration of normal cells to tumor cells will a tumor develop.

When a small block of tobacco pith tissue was isolated, planted on the basic culture medium and inoculated with crown gall bacteria, no tumors were produced. It was not surprising to find that pith cells were not altered to tumor cells under the conditions of this experiment since our earlier studies had shown that cell division or the processes leading to cell division are essential if normal cells are to be altered to tumor cells by the crown gall bacteria. Histological studies showed that cell division had not occurred at the point of inoculation or elsewhere in the pith tissue fragment. The problem then was to effect a normal wound-healing response in the pith tissue. When vascular tissue, which has been shown to be a source of both auxin and cell-division factor, was isolated with the pith tissue and the pith was inoculated with crown gall bacteria, no wound-healing reaction and no tumor formation resulted. It is known, however, that certain substances including the two growth factors of interest in this study may be translocated basipetally in the vascular region of a plant stem. When pieces of pith together with elements of the vascular system were isolated and, instead of being placed on the surface of the agar medium in their normal position as had been done in the previous experiment, were placed top-side down on the agar medium, the pith cells at the upper surface were stimulated to divide. Cell division in this instance resulted from the lateral diffusion into the pith of the two growth substances that had accumulated in the region of the vascular elements at the cut stem surface of what was originally the basal end of the tissue fragment. Inoculation of crown gall bacteria into such healing pith tissue resulted in the formation of typical crown gall tumors. Having thus found a method for inducing a normal wound-healing response in pith tissue, the above experiment was modified in such a way as to permit the vascular elements to remain in contact with the pith tissue only until the growth substances leading to cell division had diffused from the vascular region to the pith. This required about 3 days at 25°C. Thereafter the vascular tissue was removed and discarded and the pith fragments were inoculated with bacteria. Typical tumors were initiated in these experiments. The results of this study indicate that as a consequence of the alteration of normal tobacco pith cells to tumor cells, the affected cells achieve autonomy in two most essential directions. Prior to their alteration to tumor cells mature pith cells did not produce physiologically detectable amounts of either the growth factor concerned with cell enlargement or that concerned with cell division, while following their conversion to tumor cells both substances were produced. If both growth-substance-synthesizing systems had not been activated following the cellular alteration, growth by cell division and, hence, tumor formation would not have resulted in the test system used in these experiments. It nevertheless seems unlikely that the tumor-inducing principle in crown gall, which appears to be highly specific in its action, acts directly on the two biosynthetic pathways simultaneously by accomplishing either the removal of two normally occurring inhibitory systems or by effecting an increased synthesis or more efficient utilization of both growth substances simultaneously but independently by the tumor cell. A more likely explanation would appear to be that this tumor-inducing principle exerts its specific effect on one of the growth-substance systems resulting in the production of greater than regulatory amounts of that substance by the tumor cell. Concomitant with this alteration, a change is effected in the second system as a result of which the cell becomes auton-

RESPONSES OF OTHER CELLS

While normal tobacco pith cells require an externally supplied source of both the cell-enlargement and cell-division factor for growth by cell division, certain other plant cell types need only the addition of the cell-enlargement factor to the basic medium for their continued growth in culture. Such isolated cell types appear capable of synthesizing the cell-division factor. In these instances, in contrast with normal pith cells, the cell-division-factor synthesizing mechanism appears to be very lightly blocked or not blocked at all. Such cell types are readily converted to tumor cells by crown gall bacteria. This suggests that the mechanism concerned with production of cell enlargement factor is affected in some as yet unknown manner by the tumor-inducing principle, leading to an increased synthesis or decreased destruction of auxin by the cell. Since cell-division-factor synthesis appears to be normally functional in cells of this type, the presence of greater than regulatory amounts of auxin in the cells permits the continued unregulated growth of these cells. Earlier studies have demonstrated (Braun, 1953) and results similar to those reported above (Braun, 1956) have confirmed the fact that cell division or the processes leading to cell division are essential if normal cells are to be changed to tumor cells in crown gall. The normal wound-healing response may thus serve a dual function in the transformation process: 1) to make vulnerable to alteration the cellular system specifically affected by the tumor-inducing principle, and 2) to set into operation the normal processes of cell division which in the presence of greater than regulatory amounts of one of the growth factors (auxin) continue to be functional indefinitely.

STIMULATION OF SLOWLY GROWING TUMORS

If, as this work suggests, autonomy in crown gall is simply a question of the activation in tumor cells of two growth-substance systems that are more or less strongly blocked in normal cells, then it should be possible to increase the rate of growth of slowly growing benign tumors by supplementing them with either the cell-enlargement or cell-division factor or with a combination of both growth factors. Let us now consider again for a moment two of the slowly growing tumors described earlier in this discussion. When the normally small overgrowths initiated by the attenuated A66 culture on tomato are supplemented at a distance with any one of several growth substances possessing auxin activity, such tumors grow rapidly virulent strain (fig.1,C) (Braun and Laskaris, 1942; Thomas and Riker, 1948). Tomato cells altered by highly virulent strains of bacteria apparently produce, as a result of their conversion to tumor cells, high levels of available auxin. This growth substance appears, on the other hand, to be regulated at a much lower level in cells altered by the attenuated strain. In this instance the limiting factor for rapid autonomous growth seems to be an auxin.

The second system in which slowly growing benign tumors were obtained was one in which a highly virulent culture of the crown gall bacterium was allowed to act on the host cells for only 34 hours before being destroyed selectively by a thermal treatment. Tumor cells isolated from such overgrowths grew, it will be recalled, very slowly in culture when compared with the growth of cells altered by the same strain of the bacterium in a 72-hour period. When the basic culture medium on which the slowly growing tumors were planted was supplemented with either naphthalene acetic acid, a substance possessing auxin activity, or with the cell-division factor, the rate of growth of these tissues did not increase over that found when similar tissue was grown on the basic medium itself. A combination of auxin and of tumor extract containing the cell-division factor applied to the basic medium permitted some increase in the rate of growth of these tissues. Growth levels in this instance did not, however, approach that of cells altered by bacteria in a 3-day period grown on the basic medium. It is obvious from experiments of the type described above that neither the cell-enlargement nor the cell-division factor was by itself limiting for rapid growth. An additional factor or factors had therefore to be sought. Our recent studies, which are as yet preliminary, have shown that when the basic culture medium was supplemented with both yeast extract and naphthalene acetic acid, the rate of growth of the cells altered in 34 hours approximated that of cells altered by the bacteria in a 72-hour period. Neither the yeast extract nor the naphthalene acetic acid incorporated singly into the basic medium was effective in increasing the growth rate of this tissue. Since both of these factors were essential for rapid growth, the results suggest that these slowly growing tissues not only possess a requirement for some factor or factors present in the yeast extract but that the capacity for the production of available auxin in these cells was fixed at the time of their conversion to tumor cells at a level suboptimal for rapid autonomous growth. Normal plant cells altered to tumor cells in a 72-hour period are themselves capable of synthesizing optimal or near optimal concentrations of the cell division, cell enlargement, and yeast extract factors for their continued rapid and abnormal proliferation. Those altered to tumor cells in a 34-hour period, on the other hand, have had their requirements in terms of rapid growth only partially satisfied for the cell-enlargement and yeast extract factors. Thus the transition from the slowly growing benign to the rapidly growing potentially malignant crown gall tumor cell appears to be a gradual one involving quantitative rather than qualitative differences.

WOUND HEALING AND TUMOR INDUCTION

Of interest in this connection is the relationship between normal wound healing and tumor induction. An excellent correlation has been found to exist, as shown schematically in Figure 2, between the stage in the normal wound-healing cycle in which the alteration of normal cells to tumor cells is accomplished and the size and rate of growth of tumors that subsequently develop. These results, which are reported in detail elsewhere (Braun, 1952, 1954), demonstrate that a conditioning of the host cells resulting from the stimulus of a wound is essential if normal cells are to be changed to tumor cells. This conditioning process takes place gradually reaching its maximum effect between the second and third days after wounding and declines again as normal cell division begins and wound healing progresses toward completion. If the host cells are not adequately conditioned, as appears to be the case in both the early and late stages of

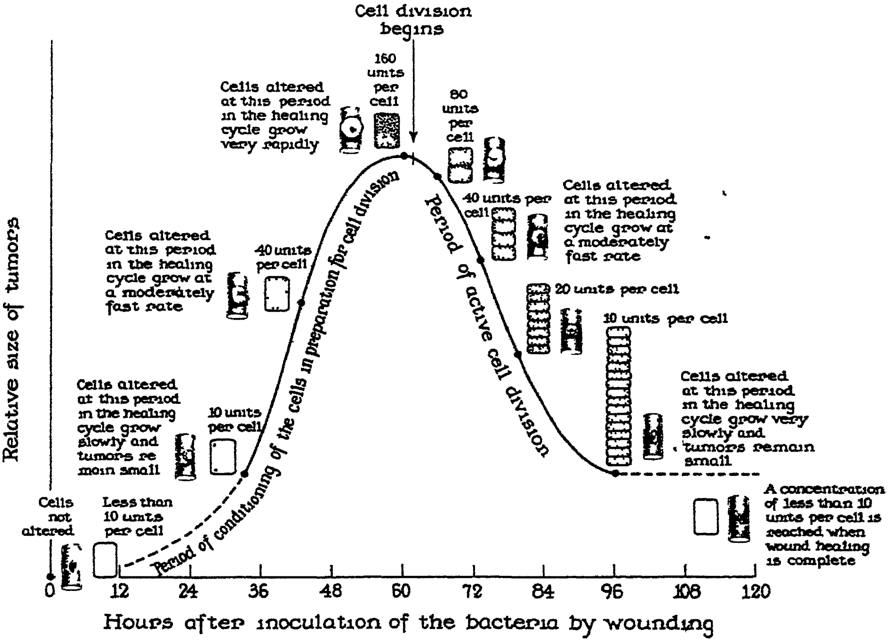

FIGURE 2. The relation of normal wound healing to tumor inception in crown gall. Conditioning of the host cells resulting from the stimulus of a wound is essential if the alteration of normal cell to tumor cell is to occur. The conditioning process, which is schematically represented, takes place gradually reaching a maximum between the second and third day after wounding and declining again as normal cell division begins and healing progresses toward completion. The size and rates of growth of the tumors produced can be correlated with the period in the normal healing cycle in which the cellular alteration is accomplished. (Photograph by J. A. Carlile)

wound healing as well as in most normal cells not under the influence of wounded tissue, then the cellular alteration will not occur despite the presence in the tissues of virulent bacteria. It thus appears that it is just before or during the earliest stages of active cell division in the normal wound-healing cycle that normal cells are most readily altered to tumor cells of the rapidly growing type. It is at this stage that the cells show a high rate of metabolic activity. Cells altered to slowly growing tumor cells in a 34-hour period are probably not fully conditioned and, hence, are not completely susceptible cells. As a result of this the cellular systems affected specifically by action of the tumor-inducing principle seem not to be as vulnerable to modification 34 hours after wounding as they are at a somewhat later period, and, hence, only a partial alteration, leading to a slowly growing benign tumor, is achieved.

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STUDIES ON HOMEOSTASIS AND HETEROZYGOSITY I. GENERAL CONSIDERATIONS. ABDOMINAL BRISTLE NUMBER IN SECOND CHROMOSOME HOMOZYGOTES OF *DROSOPHILA MELANOGASTER*.[1]

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INTRODUCTION

The classical problem of evolutionary genetics has been for a very long time that of how a population adapts to an environment. More recently, students of evolution have come to realize that the real problem is not that of adaptation to *an* environment, but rather to the multiplicity of environments in which the individual and the species exists. In effect, the species must be not only a jack-, but master-of-all-trades, if it is to succeed in competition with other forms. As has been pointed out by Dobzhansky and Wallace (1953), Thoday (1953), Waddington (1953) and many others, this wide adaptation may be accomplished either by a polymorphism, whereby the species is a heterogeneous collection of genotypes each adapted to a specific environment, or else by individual adaptability such that each organism may itself adapt to a wide variety of environments. For most species these alternatives are combined in varying degree.

Accompanying this interest in the problem of diverse adaptation has been the appearance of diverse terminologies all meant to describe some aspect of the ability of organisms and groups of organisms to adapt to varying circumstances.

As this is the first of a projected series of papers dealing with this problem, it is essential to make some nomenclatural convention, to attempt to homologize it with ideas already current, and finally to remain consistent in its use.

DEFINITION OF HOMEOSTASIS

"Homeostasis" is a term which has been used by Dobzhansky and Wallace (1953) and by Lerner (1955) in their discussion of adaptation to diverse environments. As the title of this present paper indicates, it is this term which will be adopted for our discussion as well. In attempting to define homeostasis in a genetic context, it is best to look back to its original use by Cannon (1932). The sense of Cannon's "Wisdom of the Body" is that a homeostatic organism is one which maintains *certain aspects* of its physiology constant, despite environmental forces tending to disturb this constancy. The words "certain aspects" have been italicized because they lie at the very heart of the concept of homeostasis. Homeostasis does not mean constancy of all aspects of the organism's physiology. It does not mean constancy *per se*.

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As an example one might consider the maintenance of body temperature in homoiotherms. In order to maintain temperature constant despite external fluctuation it is necessary that the organism vary its basal metabolism rate, dilation of the peripheral blood vessels, output of the sweat glands, erection and amount of body hair, and activity of various endocrine glands, as well as other structures and processes. If stability of body temperature is an indication of homeostasis, so then is the lability of the various other factors, for in any homeostatic system, stability at one level is dependent upon lability at others.

If both lability and stability are characteristic of homeostasis, how can one contrast the homeostatic adjustment of two organisms? Which of the multitude of physiological properties of a homeostatic physiology are constant, and which variable? The answer as given by Cannon is quite clear. A homeostatic organism maintains constant those aspects of its physiology whose constancy is necessary for survival. Conjugately, such an organism will be characterized by lability of those processes in which lability is adaptive. These considerations lead to the following definitions of homeostasis as used in a genetic context. A genotype is homeostatic if individuals of this genotype can so adjust their physiology and morphogenesis as to survive and leave offspring in a wide variety of environments. A population or species is homeostatic if its genotypic or phenotypic composition can be so adjusted as to assure its survival in a variety of environments.

Homeostasis, dependent as it is on adaptive value and range of environments for its definition, is a relative term by which genotypes or groups of genotypes may be compared. There is, in this definition at least, no criterion of absolute homeostasis. Moreover, we have deliberately avoided the use of the term "constancy," but refer only to the ability to adjust adaptively to varying environments. This is the essence of homeostasis, at least as we understand it.

A consequence of the foregoing definition is that a direct measure of homeostasis is the mean adaptive value of a genotype over a determined set of environments. Operationally, then, one genotype is defined as being more homeostatic than another if its mean adaptive value is higher in the specified environmental range. In actual practice the choice of environments for a test must be arbitrary and their relation to the multiplicity of environments in nature can only be guessed at.

THE FORMS OF HOMEOSTASIS

Homeostasis may be either collective or individual. An example of collective homeostasis is balanced polymorphism, whereby the continuity of a population through time is assured by the presence in the population of a variety of differently adapted genotypes. Another mechanism of collective homeostasis is found in the rapid reproductive rate of bacteria which allows rapid change in the genetic constitution of the population through mutation and selection. These modes of collective homeostasis are not to be confused with the "genetic homeostasis" described by Lerner (1954). What Lerner discusses is the property of a population to resist sudden changes in its genotypic composition. These changes would presumably be due, for the most part to the occurrence of new selective forces. Such a resistance may often be adaptive in the sense that populations which responded too readily to every short term change in selective force, might easily be trapped in an evolutionary *cul-de-sac*, and then extinguished. This would be the case if all of the genetic variation of the population were lost in a rapid response to a unidirectional selective force. On the other hand, the consequences of resistance to change may just as easily be non-adaptive. Such was the case with some of the populations described by Mather and Harrison (1953). The resistance to selection manifested by these populations resulted in their extinction. If we are to maintain any adaptive criterion of homeostasis, a property which leads to extinction cannot be included under this rubric. Perhaps this concept of Lerner's is better thought of as "genetic impedance" by analogy to that property of electrical circuits which produces both a time lag and a dampening effect on the magnitude of oscillations in an electric current which is subjected to fluctuations in electromotive force. As Lerner points out, his concept is similar to, if not identical with "genetic inertia" of Darlington and Mather (1949). It is also related to the "genetic stability" of Thoday (1953) which is simply the like-produces-like property of genetic systems. It is accomplished by the reproductive stability of the gene, by linkage, by whatever mechanisms exist to prevent the rapid appearance of new genetic variation. This stability, although anti-evolutionary in one sense, is essential to evolution. For, if genotypes do not have some continuity in time, it is impossible for natural selection to act in any directed fashion.

As evolution progresses homeostatic mechanisms tend to change from collective to individual ones. As has been mentioned, bacteria depend in large part on a rapid reproductive rate for their adaptation. This is not to say that individual homeostasis is entirely lacking in bacteria, for indeed, adaptive enzyme formation is an example of individual accomodation. As in all biological generalizations there are many exceptions. Nevertheless the overall trend, if not the chief product of evolution is individual homeostasis. This is simply the effect of stabilizing selection discussed at great length by Schmalhausen (1949).

To achieve individual homeostasis an organism has two usually conflicting forces to deal with. During its morphogenesis it must adapt to whatever forces impinge upon it, yet at the same time it must have the ability to produce an adult morphology and physiology capable of adapting to conditions which may not have been present during its embryonic life. The organism may meet the challenge of fluctuating environment during morphogenesis by a diversity of morphogenetic pathways, each of which is adaptive in a particular environment. Should such a system of alternatives exist, however, the end product of many of them might be an ill-adapted adult. Waddington's "canalization" (1953) is the process of restriction of the pathways in order to assure the adult of an adaptive morphology. As such, it is a solution to only half the problem for in its effort to do or die, the embryo may die. "Developmental homeostasis" (Lerner, 1954) and "developmental flexibility" (Thoday, 1953) seem to cover both of these processes. Thus Thoday writes:

> "An individual may be said to possess developmental flexibility either....if it can develop different phenotypes in different environments, each phenotype better adapted than the others to the environment that evokes it, or if its....development is buffered against environment variables and hence apparently the same adaptive phenotype results in a range of environmental conditions."

The first alternative is that of the adaptation of the embryo, the second, of the adult. This does not mean that a choice between these alternatives exists. An organism, if it is to be homeostatic, must do both. A simple example will illustrate this conflict. If Drosophila larvae are grown under starvation conditions, they will produce a fair number of quite small pupae, which in turn eclose as small adults. The production of these smaller than normal flies is an adaptive response to the lack of food. However, these diminutive adults are not fully fertile. Whether it would be of advantage to produce very few, fully fertile adults, rather than a larger number of smaller, semi-sterile individuals can only be answered in a specific case. But clearly adaptation in embryonic and post-embryonic life are opposed in this case.

It is this general problem to which both intra-uterine development in mammals and persistent meristematic tissue in plants, are solutions.

VARIABILITY AS A MEASURE OF HOMEOSTASIS

It might be supposed that since homeostasis involves constancy, at least by implication, that some measure of variability might serve as a criterion of homeostasis. In fact no measure of variability alone is sufficient. That this is so may be seen by considering variation of two sorts, one in physiological and morphological traits, the other in adaptive value itself.

The ambiguity involved in using morphological or physiological variation as an index of homeostasis has already been discussed. Some characteristics of a homeostatic physiology vary while others remain constant. Moreover, this relationship is a causal one. In any system of dynamic equilibrium the constancy of one feature of the system is made possible only by variation in other features. Without some previous information as to the adaptive significance of variation in a given character, the existence of lability or stability *per se* can give no information on homeostasis. Presumably if such previous information did exist, a measure of variability would be pertinent. Ultimately, however, such information can come only from actual experiment on the adaptive consequences of variation in the character. Ultimately, then, the characterization of a genotype as homeostatic is dependent upon the observation of its adaptive value in a variety of environments.

Both Lerner (1954) and Thoday (1953) point out that only characters of adaptive significance ought to be used in measuring homeostasis. Despite this, both authors make abundant use of evidence on the relative variance in wing length and bristle number in Drosophila as evidence that heterozygotes are more homeostatic than homozygotes. Moreover, Lerner, when citing evidence based upon characters of obvious adaptive significance, does not make the important distinction between characters which are components of adaptive value and those whose *variation over a range of environments* is a component of adaptive value. Thus egg weight in poultry is undoubtedly of adaptive significance, but whether it is of advantage to have egg weight vary with environment or remain constant is not clear. Indeed, arguments might be advanced for either alternative.

Turning to variation in adaptive value itself, the situation is somewhat more complex. The relation between mean and variance in adaptive value can best be seen by the following artificial example. Six different genotypes are tested in four environments. Their relative adaptive values in each environment are recorded in table 1 along with the average adaptive value, $\overline{W}$, and the variance in adaptive value, σ^2_W, for each genotype. We may assume that the genotypes are equally frequent in each environment, or else each adaptive value may be weighted by the probability that a genotype will be found in a particular environment. This would account for cases in which individuals actually sought out environments to which they were best adapted. Using mean adaptive value as the criterion of homeostasis, the relative homeostasis of the genotypes in table 1 would be

$$A > B > C > D = E > F$$

On the other hand if it were assumed that low variance in adaptive value signified greater homeostasis, the order of the genotypes would be

$$F = D = B > E > C = A$$

which is clearly not consonant with the first ordering. That a comparison based upon constancy alone is unsatisfactory is obvious, for such a system would assign to genotype F a high value of homeostasis. No definition which terms a lethal genotype homeostatic is admissible for it completely traduces the meaning of this concept. An ordering based upon mean adaptive value, however, avoids such difficulties although it leads to a result which at first sight seems unorthodox. Thus, under our definition, genotype A is more homeostatic than genotype B despite the higher variance of the former. In addition genotypes D and E are equally homeostatic although they possess quite different variances in adaptive value. Such a result may be disquieting but if homeostasis is to be any measure of the evolutionary future of a genotype, of the capacity of a genotype to

TABLE 1

POSSIBLE RELATIVE ADAPTIVE VALUES OF 6 GENOTYPES TESTED IN 4 ENVIRONMENTS. $\bar{W}$ IS THE MEAN ADAPTIVE VALUE AND σ^2_W IS THE VARIANCE OF ADAPTIVE VALUES.

	Environments					
Genotypes	I	II	III	IV	$\bar{W}$	σ^2_W
A	7	4	4	2	4.25	4.25
B	4	4	4	4	4	0
C	6	4	4	1	3.75	4.25
D	3	3	3	3	3	0
E	5	3	3	1	3	2.67

adapt on the average to varying environment, no other result seems permissible.

Dobzhansky and Wallace (1953) have studied the variance in viability of genotypes homozygous or heterozygous for the gene contents of various chromosomes in several species of Drosophila. In their summary they state:

> "The homozygotes often show significantly different survival rates in the varying environments of the replicate cultures. Conversely, the heterozygotes gave usually uniform survival despite the environmental variations. It is inferred that the homeostatic adjustments are superior than in homozygotes."

On the basis of this statement it would seem that the criterion of homeostasis which these authors have used is that of variance rather than of mean adaptive value. Nevertheless, what is significant in their data is that heterozygotes show higher mean adaptive values as well as lower variances. Their conclusions about homeostasis are actually based upon this association. If such an association were the rule, no conflict would exist between mean and variance as criteria of homeostasis. In terms of the artificial example described above, genotypes would be either of type B or of type E. That this association is not the rule, is clear from two considerations. First, lethals have both the lowest possible mean adaptive value and the lowest possible variance in adaptive value. Ignoring this objection as being simply a special case, a second objection is apparent when some of the data upon which Dobzhansky and Wallace based their conclusions is examined. Figure 1 is based upon data taken from Dobzhansky and Spassky (1944) on the viabilities of fourth chromosome homozygotes in *Drosophila pseudoobscura.* The viability of these homozygotes was tested at three temperatures. The abcissa in figure 1 represents the mean viability of a given genotype over the three temperatures, while on the ordinate are plotted the values of chi-square as a measure of the varia-

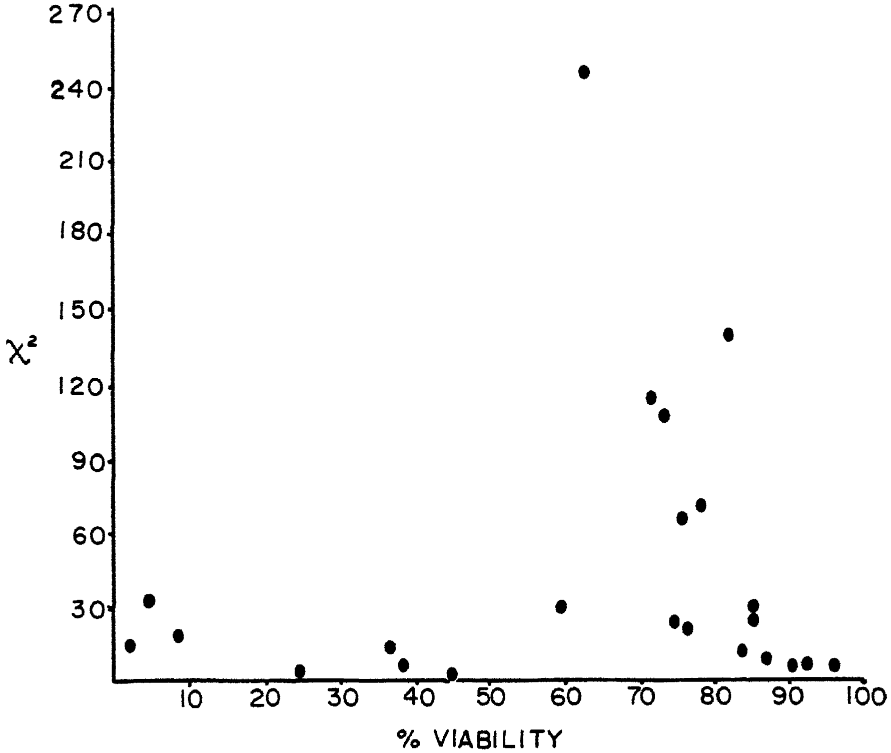

FIGURE 1. Scatter diagram showing the relation of mean viability over three temperatures to the χ^2 between temperatures in fourth chromosome homozygotes of *D. pseudoobscura*. Data taken from Dobzhansky and Spassky (1944).

tion in viability from temperature to temperature. Viability is expressed as per cent of normal; normal being defined as the mean viability of heterozygotes. The points show that small variation is associated both with high and with low mean value. At intermediate viabilities, both high and low values of χ^2 are obtained. The two lowest values of χ^2 are not associated with high viability at all, but with mean values of 45 per cent ($\chi^2_2 = 0.7$) and 24 per cent ($\chi^2_2 = 2.6$). This is a graphic demonstration that there is no one to one correspondence between viability and variance.

There is no conflict between this result and the conclusions of Dobzhansky and Wallace. A comparison of figure 1 with figure 2 shows why this is so. This latter curve is based upon data taken from Dobzhansky and Spassky (1954). It shows the frequency distribution of the viabilities of a random sample of homozygotes for the second chromosome of *Drosophila prosaltans*, as compared to that of heterozygotes. The general pattern of this distribution is not confined to this particular chromosome or species, but is repeatedly found whenever a similar test is performed, at least in Drosophila. Heterozygotes are confined to that region of the distribution where there is both high mean viability and low variance.

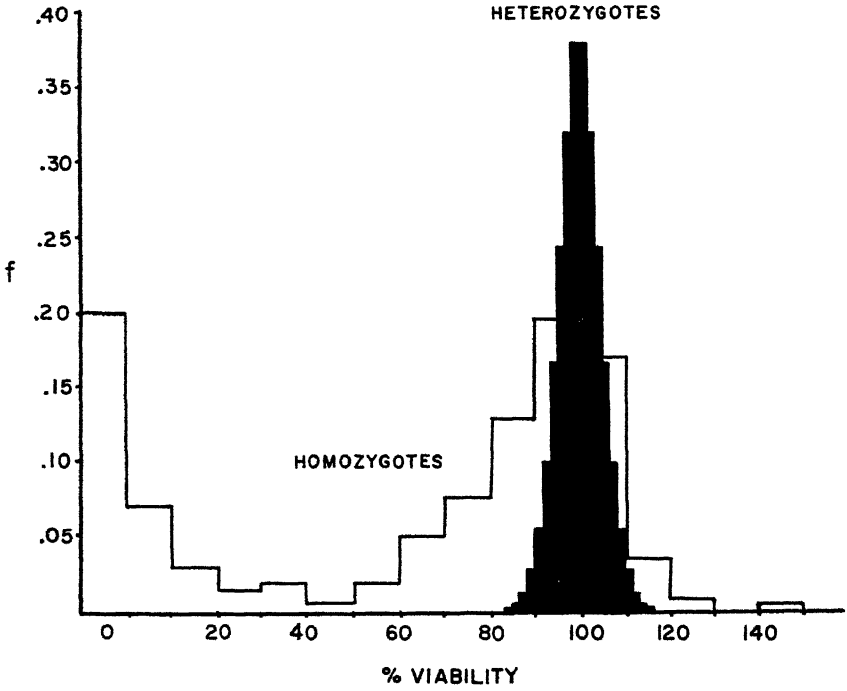

FIGURE 2. Frequency distribution of viability for second chromosomes of *D. prosaltans*. Homozygotes: open bars; heterozygotes: solid bars. Data from Dobzhansky and Spassky (1954).

Homozygotes, on the other hand, have not only a lower mean viability, but are concentrated in regions where both high and low variances exist. Presumably, if heterozygotes could be found which had lower mean viabilities, some of them would show higher variances just as do homozygotes. More important is the fact that homozygotes with high mean viabilities show variances quite as small as do heterozygotes. Small variance in viabilities is not confined to heterozygotes, nor does it give any index of the average fitness of a genotype for it may be observed at any value of viability from lethal to supervital.

The lack of high values of χ^2 in the region between 10 and 50 per cent mean viability makes any theoretical explanation of the observations in figure 1 quite difficult. What is required now is information on a large number of homozygotes covering the entire range of viabilities.

To recapitulate, the lack of one to one correspondence between average fitness and variance of fitness makes it impossible to use this variation as a measure of homeostasis. Rather, a high average fitness itself is a necessary and sufficient criterion of homeostasis. In our view it is the only valid one. In addition, variance in a morphological or physiological character cannot be taken, *per se*, as a measure of homeostasis, but must be coupled with a knowledge of the adaptive meaning of such variation.

It is this problem of the relation between morphological variation and homeostasis which we shall investigate in this and following papers.

MATERIALS AND METHODS

Eighteen strains of *D. melanogaster* homozygous for sub-vital or semi-lethal gene complexes were used in this experiment. In deriving the strains from files maintained in population cages, each was made homozygous for a different second chromosome by a method described by Wallace (1952). We are indebted to Dr. Wallace and his collaborators for providing samples from their populations. The strains were characterized as to their egg hatchability and larval viability under a variety of population conditions. The results of these tests are described by Lewontin (1955). The numbering of the strains in the present paper is consistent with the previous publication to facilitate comparison.

From the eighteen homozygous strains, eighteen F_1 strains were derived by the following cyclical design. If the homozygous lines are designated A, B, C, ... R, the crosses are made

♂♂ A × ♀♀ B
♂♂ B × ♀♀ C

♂♂ Q × ♀♀ R
♂♂ R × ♀♀ A

Each homozygous line enters two F_1 crosses, once as a male parent and once as a female. Each F_1 was then mated *inter se* to produce an F_2 generation. Five replicate cultures of each homozygote and each cross were obtained by serial transfer of a single group of parents every 48 hours. After 48 hours in the last bottle the parents were discarded. No particular attempt was made to standardize replicate bottles as some environmental variation was desired. All cultures were kept at 20° ± 0.5°C.

From each replicate ten males and ten females were examined and the number of bristles on the fourth and fifth sternites were counted. No individual placed in position for counting was rejected except a few cases where tumors or an abnormal abdomen distorted the bristle bearing area. In this way unconcious selection on the basis of bristle number was avoided.

It should be pointed out that the homozygote, F_1 and F_2 series were made from three to four weeks apart and thus were not strictly simultaneous. Within each set, however, all strains were transferred simultaneously for replications. These replications covered a period of ten days and actual examination covered the full three to four weeks between sets. Thus, it is not likely that temporal changes in experimental conditions from the beginning to the end of the experiment contributed to the differences observed between homozygotes, F_1 and F_2. It will be seen that the consistency of results bears out this point.

ESTIMATES OF VARIANCE

What is being sought in this experiment is the effect of varying environments on the phenotype of the strains investigated. A direct measure of this effect is the variance in bristle number apportionable to environmental differences. There are several ways in which this environmentally determined variance can be estimated. First, the variance between individuals within replicate cultures may be used. Designating this variance as σ^2_w, it will be compounded of the following elements:

$$\sigma^2_w = \sigma^2_G + \sigma^2_e + \sigma^2_{Ge} + \sigma^2_r + \sigma^2_{re} \tag{1}$$

where σ^2_G = genetic variance due to segregation on the second chromosome
σ^2_e = variance due to micro-environmental variation
σ^2_r = genetic variance due to segregation of the residual genotype

and σ^2_{Ge} and σ^2_{re} are the interaction variances between the appropriate factors. Now for homozygotes and F_1 crosses σ^2_G will be zero. Moreover, the method of extracting the second chromosome is such that the residual genotypes are on the average the same for all strains and between crosses of the same strain. This being so, one can ignore the last two terms in expression (1) when comparing variances of different strains or crosses. The only two terms left are the micro-environmental variance and the interaction variance between genotype and micro-environment, these being the components of interest.

A second estimate may be derived from the variance between means of the five replicates in each cross. Designating this variance as σ^2_b we see that

$$\sigma^2_b = \sigma^2_E + \sigma^2_{GE} + \frac{1}{n}\sigma^2_w \tag{2}$$

where σ^2_E = variance due to differences in environment between replicates and n = number of individuals measured in each bottle.

Since n is known and we have already estimated σ^2_w, we have only to substitute them in (2) to get an estimate of the effects of bottle-to-bottle variation in environment and its interaction with the genotype.

A third variance estimate of interest is of a somewhat different nature. For any individual measured, segments four and five in general have different numbers of bristles, segment five on the average more than segment four. In any individual this gradient may be reversed. Using the replication means as a measure of the average difference between the two segments, the variance of this difference from individual to individual is calculated. This variance is approximately related to the correlation $r_{4,5}$ between these segments by the expression

$$\sigma^2_d \cong (\sigma^2_4 + \sigma^2_5)(1 - r_{4,5}) \quad \text{when} \quad \sigma^2_4 \cong \sigma^2_5 \tag{3}$$

We have chosen to use σ^2_d rather than $r_{4,5}$ because the former increases with increasing effect of environment as do the other two variance estimates, while the correlation changes in the opposite sense. In addition $r_{4,5}$ depends upon the variance in total bristle number while σ^2_d is free of the effects of this variance. Thus, σ^2_d can be compared with less confusion. It is simply a measure of the lack of correlation between the two segments of an individual.

RESULTS

Tables 2, 3, and 4 show the mean number of bristles and the variance components just described for homozygous, F_1 and F_2 crosses. Males and females are tabulated separately since they have quite different average bristle numbers. The values of σ^2_w and σ^2_d represent pooled estimates

TABLE 2

MEAN ABDOMINAL BRISTLE NUMBER AND VARIANCE COMPONENTS FOR HOMOZYGOTES, F_1 AND F_2 IN MALES. SEE TEXT FOR EXPLANATION OF SYMBOLS.

Strain	$\bar{X}$		σ^2_w		σ^2_b		$\sigma^2_E + \sigma^2_{GE}$	
	Hom. & F_1	F_2	Hom. & F_1	F_2	Hom. & F_1	F_2	Hom. & F_1	F_2
1	36.28		10.09		2.65		1.64	
1 x 11	35.26	36.78	12.80	7.98	3.25	1.85	1.97	1.05
11	35.58		5.58		.71		.16	
11 x 18	34.34	34.58	11.31	8.18	2.36	.71	1.23	-.11
18	34.44		4.29		.41		-.02	
18 x 22	31.56	33.62	9.89	5.58	3.92	.38	2.93	-.18
22	30.10		7.39		2.14		1.40	
22 x 25	31.96	30.60	7.49	6.47	2.93	.83	2.18	.18
25	32.36		6.07		1.69		1.08	
25 x 27	33.86	31.28	7.18	5.47	1.87	4.44	1.16	3.90
27	32.18		5.47		1.34		.80	
27 x 28	35.22	33.74	10.53	12.93	1.23	1.24	.17	-.06
28	35.10		10.98		1.06		-.04	
28 x 33	36.26	34.08	10.49	5.82	1.49	.75	.44	.17
33	34.76		6.73		.84		.17	
33 x 34	33.84	34.90	5.80	10.00	1.98	1.45	1.40	.45
34	32.84		6.73		1.38		.71	
34 x 35	34.06	33.66	7.40	6.31	.40	.64	-.34	.01
35	35.06		5.47		.99		.45	
35 x 41	34.14	33.52	8.09	15.42	1.80	2.70	.99	1.16
41	31.84		5.96		1.89		1.30	
41 x 45	32.74	34.30	9.13	5.36	.32	3.56	-.59	3.03
45	34.48		5.29		.34		-.19	
45 x 47	33.04	33.92	4.56	4.53	3.42	3.48	2.97	3.03
47	32.26		5.04		.32		-.18	
47 x 48	34.98	32.80	8.78	9.04	2.64	.69	1.77	-.22
48	32.40		8.80		4.85		3.97	
48 x 54	35.22	32.48	9.87	8.80	.42	.65	.57	-.23
54	32.20		8.32		.44		-.40	
54 x 62	35.06	34.32	6.07	7.33	.64	1.02	.03	.29
62	34.96		6.91		1.12		.43	
62 x 67	33.72	32.42	5.22	9.04	3.38	1.19	2.86	.28
67	33.00		8.32		2.59		1.75	
67 x 74	34.94	34.42	6.31	9.24	.52	.84	.11	-.08
74	35.90		7.88		.71		-.08	
74 x 1	34.36	34.60	8.18	6.36	1.99	4.90	1.17	4.27
1	36.28		10.09		2.65		1.64	

TABLE 3

MEAN NUMBER OF ABDOMINAL BRISTLES AND VARIANCE COMPONENTS FOR HOMOZYGOTES, F_1 AND F_2 IN FEMALES. SEE TEXT FOR EXPLANATION OF SYMBOLS.

Strain	$\overline{X}$		σ_w^2		σ_b^2		$\sigma_E^2 + \sigma_{GE}^2$	
	Hom. & F_1	F_2	Hom. & F_1	F_2	Hom. & F_1	F_2	Hom. & F_1	F_2
1	45.02		10.76		5.15		4.07	
1 x 11	43.62	45.98	17.18	20.22	9.53	3.63	7.81	1.61
11	43.28		10.80		2.01		.93	
11 x 18	41.92	43.28	11.29	12.58	4.94	.25	3.81	-1.01
18	42.06		6.87		.57		-.11	
18 x 22	39.52	42.26	1.49	12.62	.51	1.34	.36	.08
22	38.02		5.04		2.70		2.20	
22 x 25	38.82	37.54	11.04	7.20	2.97	.92	1.86	.20
25	37.06		9.11		.83		-.08	
25 x 27	39.80	38.52	9.36	9.24	.77	.35	-.17	-.57
27	40.80		8.20		.45		-.37	
27 x 28	48.86	42.20	9.53	16.87	2.82	3.47	1.87	1.78
28	43.20		9.31		3.42		2.48	
28 x 33	45.20	41.96	14.44	14.98	.42	2.20	-1.03	.70
33	42.54		8.40		3.35		2.51	
33 x 34	41.30	40.92	17.44	14.36	.74	1.90	-1.00	.46
34	39.06		10.64		3.84		2.77	
34 x 35	38.89	40.22	9.47	7.09	.73	.94	-.22	.23
35	40.74		10.40		2.28		1.24	
35 x 41	40.20	40.04	8.67	13.89	4.25	.82	3.38	.57
41	39.24		8.00		1.26		.46	
41 x 45	41.18	44.14	6.63	13.64	1.04	2.45	.40	1.09
45	43.22		6.87		3.69		3.00	
45 x 47	40.80	40.76	5.78	13.64	3.00	7.83	2.42	6.46
47	39.16		4.71		.52		.05	
47 x 48	44.08	42.32	9.93	22.07	2.26	2.85	1.27	.65
48	39.92		9.29		3.41		2.48	
48 x 54	43.40	41.30	16.96	13.33	1.08	3.11	-.62	1.77
54	39.46		10.80		1.63		.55	
54 x 62	41.26	42.04	15.84	20.64	5.02	3.03	3.44	.96
62	42.50		13.64		2.89		1.53	
62 x 67	41.04	39.02	10.82	11.49	4.83	1.11	3.75	-.04
67	39.34		8.00		5.08		4.28	
67 x 74	41.34	42.96	20.49	14.20	1.39	2.02	-.66	.60
74	44.44		11.34		1.21		.08	
74 x 1	44.04	43.40	17.62	11.16	6.97	3.11	5.21	1.99
1	45.02		10.76		5.15		4.07	

from the five replications. Table 5 shows a condensed comparison among the homozygous, F_1 and F_2 crosses, the entries in which are simply the grand totals of the appropriate columns in tables 2, 3, and 4.

The first thing which is clear from table 5 is that there is little or no heterosis in the sense used in quantitative genetics ("luxuriance" in the terminology of Dobzhansky, 1952). The F_1 crosses show a very slight, nonsignificant increase in mean bristle number over the homozygotes. The F_2 values drop back again, in the case of males to the parental value, in the case of females to an intermediate one. The genes seem to be acting in an additive fashion with perhaps a small amount of dominance.

Inspection of the variances shows a more interesting picture. In both sexes σ_w^2 and $\sigma_E^2 + \sigma_{GE}^2$ are higher in F_1 than in homozygotes. On the other hand σ_d^2 is lower for heterozygotes. Table 6 shows the value of the variance ratios (F) for the various comparisons along with the appropriate degrees of freedom and probability levels. The F values shown for comparing the values of $\sigma^2_E + \sigma^2_{GE}$ have been calculated in a special way. Because they are derived by subtracting one estimate of variance from another they cannot be tested by the usual F ratio. Instead we have used a method suggested by Cochran (1951) to which Mr. Gilbert Paul has drawn our attention. The ratio

TABLE 4

VARIANCE OF THE DIFFERENCES BETWEEN SEGMENTS 4 AND 5 (σ^2_d) IN HONOZYGOTES, F_1 AND F_2 MALES AND FEMALES.

	Males			Females		
Strain	Hom.	F_1	F_2	Hom.	F_1	F_2
1	4.76			6.93		
		3.73	4.16		4.29	5.11
11	4.91			4.89		
		4.16	6.44		4.89	6.89
18	4.16			5.44		
		4.24	3.89		4.31	3.56
22	2.25			3.93		
		5.53	5.18		5.27	5.11
25	4.02			6.36		
		2.87	4.53		5.27	4.49
27	3.87			5.13		
		5.56	4.09		4.69	6.51
28	6.69			5.71		
		3.64	4.84		4.78	4.67
33	5.22			5.78		
		4.51	3.02		6.11	2.93
34	5.58			7.18		
		3.67	3.64		3.47	5.84
35	6.80			5.56		
		4.31	5.29		4.00	4.51
41	3.82			7.38		
		4.11	2.47		4.62	4.40
45	3.29			5.67		
		5.62	4.13		5.38	4.36
47	3.76			6.62		
		3.67	3.13		5.67	5.89
48	3.29			9.47		
		3.73	3.64		5.89	5.33
54	4.82			12.07		
		2.96	6.18		8.96	6.64
62	3.44			6.13		
		3.00	3.62		3.93	4.20
67	4.09			4.22		
		5.51	2.67		4.18	4.60
74	6.53			8.39		
		2.18	6.62		4.47	6.49
1	4.76			6.93		

TABLE 5

CONDENSED COMPARISONS OF HOMOZYGOTES, F_1 AND F_2. ENTRIES ARE TOTALS FROM TABLES 2, 3, AND 4.

		$\overline{X}$	σ^2_w	σ^2_b	$\sigma^2_E+\sigma^2_{GE}$	σ^2_d
Males	P	606.10	125.32	25.47	12.93	81.30
	F_1	614.56	140.92	34.56	19.67	72.98
	F_2	606.02	143.87	31.32	16.93	77.55
Females	P	739.06	162.18	44.30	28.07	116.86
	F_1	756.27	213.98	53.25	31.88	90.16
	F_2	748.86	249.21	41.31	16.38	91.53

$$F' = \frac{\sigma^2_{b1} + \frac{1}{10}\sigma^2_{w2}}{\sigma^2_{b2} + \frac{1}{10}\sigma^2_{w1}} \tag{4}$$

is used. This has degrees of freedom

$$\frac{\left[\sigma^2_{b1} + \frac{1}{10}\sigma^2_{w2}\right]^2}{\frac{(\sigma^2_{b1})^2}{N_1} + \frac{\left(\frac{1}{10}\sigma^2_{w2}\right)^2}{N_2}} \quad \text{and} \quad \frac{\left[\sigma^2_{b2} + \frac{1}{10}\sigma^2_{w1}\right]^2}{\frac{(\sigma^2_{b2})^2}{N_1} + \frac{\left(\frac{1}{10}\sigma^2_{w1}\right)^2}{N_2}} \tag{5}$$

in the numerator and denominator, respectively. N_1 and N_2 are the number of degrees of freedom for σ^2_b and σ^2_w.

As table 6 shows, σ^2_w is significantly higher in heterozygotes than in homozygotes. σ^2_b and $\sigma^2_E + \sigma^2_{GE}$ are also higher but not significantly so. This is presumably due to the much smaller number of degrees of freedom available for these comparisons, with a resulting decrease in sensitivity of the test. σ^2_d on the other hand, is significantly lower in F_1 than in the parents. In the F_2, the within-bottle variances are even higher than in F_1. This is to be expected when it is remembered that the F_2 is the first generation in which genes on the second chromosome are segregating. Despite this increase, however, the values of σ^2_b are lower than in the F_1. The result is a marked, consistent, but not significant decrease in the estimate of $\sigma^2_E + \sigma^2_{GE}$. Finally the F_2 values of σ^2_d are intermediate in value between homozygotes and F_1.

There is one other item of information which is pertinent to this study. As has been mentioned there is an average anterior-posterior gradient in bristle number, the posterior segment generally having more bristles than the anterior. Table 7 shows the mean difference between these segments expressed as (posterior-anterior). For heterozygotes, all of these differ-

TABLE 6

F-RATIOS, DEGREES OF FREEDOM AND PROBABILITY LEVELS FOR COMPARISONS OF HOMOZYGOTES, F_1 AND F_2 VARIANCES IN TABLE 4.

			Males			
			σ_w^2	σ_b^2	$\sigma_E^2+\sigma_{GE}^2$	σ_d^2
$P - F_1$	F		1.12	1.36	1.19	1.11
	d. of	num.	810	72	132	810
	freedom	den.	810	72	169	810
	Pr.		.05	.10	.10-.20	.05-.10
$F_1 - F_2$	F		1.02	1.10	1.08	1.06
	d. of	num.	810	72	142	810
	freedom	den.	810	72	149	810
	Pr.		$>.20$	$>.20$	$>.20$	.20
$P - F_2$	F		1.15	1.23	1.10	1.05
	d. of	num.	810	72	139	810
	freedom	den.	810	72	171	810
	Pr.		.01-.05	.10-.20	$>.20$	$>.20$
			Females			
			σ_w^2	σ_b^2	$\sigma_E^2+\sigma_{GE}^2$	σ_d^2
$P - F_1$	F		1.32	1.20	1.06	1.30
	d. of	num.	810	72	121	810
	freedom	den.	810	72	155	810
	Pr.		$<.001$	.10-.20	$>.20$	$<.001$
$F_1 - F_2$	F		1.17	1.29	1.25	1.02
	d. of	num.	810	72	152	810
	freedom	den.	810	72	161	810
	Pr.		.01-.05	.10-.20	.05-.10	$>.20$
$P - F_2$	F		1.54	1.07	1.20	1.28
	d. of	num.	810	72	171	810
	freedom	den.	810	72	137	810
	Pr.		$\ll.001$	$>.20$	.10	$<.001$

TABLE 7

MEAN DIFFERENCE IN ABDOMINAL BRISTLE NUMBER BETWEEN SEGMENTS 4 AND 5 EXPRESSED AS (5-4) IN HOMOZYGOTES, F_1 AND F_2 CROSSES.

Strain	Males			Females		
	Hom.	F_1	F_2	Hom.	F_1	F_2
1	3.4			1.5		
1 x 11		4.3	4.9		4.3	2.3
11	2.5			3.5		
11 x 18		6.1	3.7		3.8	4.0
18	5.6			3.2		
18 x 22		5.4	4.9		5.2	.3
22	3.4			3.5		
22 x 25		8.0	3.2		4.5	4.3
25	6.2			2.1		
25 x 27		5.5	3.4		1.8	2.4
27	1.7			-.6		
27 x 28		2.3	1.1		3.3	2.8
28	.6			2.2		
28 x 33		6.3	3.2		5.6	5.2
33	1.2			3.9		
33 x 34		4.8	4.7		3.9	2.8
34	3.6			7.7		
34 x 35		3.9	3.9		1.6	2.5
35	.3			.5		
35 x 41		4.1	4.8		4.4	3.4
41	2.2			6.3		
41 x 45		4.1	3.3		4.9	1.3
45	5.0			2.9		
45 x 47		5.0	1.6		4.8	.8
47	3.5			1.4		
47 x 48		3.9	1.6		3.8	.6
48	-1.6			-.6		
48 x 54		4.1	2.4		5.6	1.9
54	-.7			3.0		
54 x 62		3.3	4.6		2.9	.2
62	4.6			-.1		
62 x 67		5.4	4.9		1.2	2.9
67	1.8			3.6		
67 x 74		8.5	3.3		3.3	2.6
74	3.4			5.3		
74 x 1		7.8	3.6		5.4	-1.2
1	3.4			1.5		
σ^2	3.93	2.96	1.35	5.11	1.96	2.39

ences are positive and fairly uniform. For homozygotes, however, some are negative and they are considerably more variable. If the values for the variances are pooled for males and females, homozygotes have a significantly higher variance than heterozygotes (P = .05). If the individual values for replicate cultures are examined the same phenomenon appears.

Among 180 homozygous cultures there are 38 negative differences, while among a like number of heterozygous cultures there are only 11, the F_2 being intermediate with 31 negative values. Homogeneity tests on the number of negative and positive values for the three types of crosses show the F_1 to be significantly different from the homozygotes and F_2 ($P < .001$ in both cases) while the F_2 does not differ significantly from the homozygotes ($P = .25$).

In summary, heterozygotes show a higher variance between individuals than do homozygotes, but a lower variance, or higher correlation, between segments. The F_2 is generally intermediate between the homozygotes and F_1 except for the variance within bottles which contains genetic variance due to segregation on the second chromosome.

DISCUSSION

As has been previously stated, the purpose of this investigation has been to examine the relationship between homeostasis and morphological variation. The homeostatic properties of the various genotypes used in these experiments have already been established, both in the initial isolation of genotypes and in a more detailed analysis of their viability and egg-hatchability values by Lewontin (1955). The various homozygous strains are emphatically lacking in homeostatic properties when compared with heterozygotes. Reference to the experiments just cited shows clearly that for the two important components of adaptive value measured, homozygotes are inferior under a wide variety of population densities. By now it is well established, for Drosophila at least, that chromosomal homozygotes are with few exceptions lethal, semi-lethal, or sub-vital as compared with heterozygotes. (Dobzhansky, Holz and Spassky, 1942; Dobzhansky and Spassky, 1943; Wallace, 1952; and Wallace and Madden, 1953).

Accepting then, the greater homeostasis of heterozygotes, we may ask what the morphological correlates of this condition are. The experiments described here show that there is no simple relation between homeostasis and morphogenetic uniformity. If variance in total bristle number is used as a criterion of morphogenetic uniformity, heterozygotes must be considered as more sensitive to environmental variation. Both σ^2_W and $\sigma^2_E + \sigma^2_{GE}$ which are measures of environmentally caused variation are higher in the F_1 than in the homozygotes. On the other hand if the phenotypic correlation between parts of an organism is considered, we find it to be greater in the homeostatic heterozygotes than in the non-homeostatic homozygotes. In our experiments the anterior-posterior gradient is considerably more constant for heterozygous genotypes than for their homozygous parents. Thoday (1953) in his very interesting analysis suggests that just such a relation ought to exist. His main argument is that organisms may adapt to varying environments by varying their phenotype adaptively, but that they must, in the process, maintain a certain harmonious balance between their component parts. This is a tempting concept, but it loses force in the transfer from the experiments he cites, to those with which we are dealing. Thoday was mainly concerned with bilateral symmetry. It might be argued that it is difficult to imagine many environments in which an asymmetrical Drosophila is at an advantage when compared with his symmetrical brothers. However, in many organisms it is precisely the asymmetrical condition which has been established by natural selection. When one deals with an anterior-posterior gradient as we are doing, it is even more doubtful that homeostasis will be accompanied always by a consistent gradient. If variation in total bristle number is an adaptive response why not variation in gradient as well? Much of evolution is concerned with shape changes, such changes being due simply to alterations in morphogenetic gradients.

Our result seems to be in conflict with that of Reeve and Robertson (1954) who studied variance of bristle number in inbred lines and crosses between lines. Their conclusion was that the correlation between segments and the total environmental variance did not differ from inbred lines to heterozygotes. They make a point of the fact, however, that their experiment was performed under "optimal" conditions, which may be the key to the conflict between the results. As they show, under "optimal" (noncrowded) conditions there is very little correlation between the segments, holding genotype constant. When population density was made variable, however, there was a significant correlation between segments. Unfortunately, this part of the experiment was performed only on the heterozygotes, so that no comparison with their inbred lines is possible. It is of course entirely reasonable that under more or less uniform optimal conditions any differences in morphogenic sensitivity to environment will be minimized. The question of morphogenetic stability and homeostasis arises only when there is a multiplicity of environments.

Despite these reservations our experiments do support the following general mode of adaptation. There is phenotypic variability with the maintenance of harmonious proportion between parts of the same organism. For other characters or other organisms the relation may be quite different.

SUMMARY

Homeostasis has been defined in this paper as the property of a genotype or collection of genotypes which allows it to respond adaptively to a wide variety of environments. The measure of homeostasis is then the average adaptive value over a range of environments.

The variance in abdominal bristle number of a series of strains of *Drosophila melanogaster* homozygous for semi-lethal and sub-vital gene complexes has been examined. It is shown that these homozygotes which are less homeostatic than heterozygotes constituted from them, show a lower variance in total bristle number than do the heterozygotes or F_2 populations. On the other hand both the heterozygotes and F_2 populations show a higher correlation in bristle number between the two segments examined. It is suggested in conclusion that no one-to-one correspondence exists between phenotypic variance and homeostasis, although a higher pheno-

typic correlation between two parts of the same organism might be more often than not a concomitant of superior homeostatic adjustment.

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RECIPROCAL DEPENDENCE OF ECTODERM AND MESODERM DURING CHICK EMBRYO LIMB DEVELOPMENT

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INTRODUCTION

The limbs of vertebrates start their development as simple structures consisting of two components: a mesodermal thickening of the somatopleure and a covering of ectoderm. For a considerable period our best information about the relative parts played by these components has come from experiments with amphibian embryos. The investigations of R. G. Harrison and his students have revealed, in the words of Nicholas (1955), that, "The mass of evidence points to the determination of the limb mesoderm of urodeles as the positive factor of limb formation." The experiments on which such a statement are based are those in which limbs have developed after the mesoderm (either in situ or when grafted to a foreign site) has been covered by non-limb ectoderm. When such experiments (Harrison, 1931) were done on *Amblydyoms punctatum* embryos it was found that, with the exception of head ectoderm from neurula and post-neurula stages, all ectoderm from embryos as old as tail bud stages could support normal limb development. These limbs conformed in all respects to the "determiners" of the mesoderm. Only such minor features as digit shape could be ascribed to an ectodermal influence when there was an exchange of components between species (Rotmann, 1933).

Not all of the evidence, even among the amphibia, is consistent with these ideas. Balinsky (1935) found that there was a limited distal development when Triton limb buds were grown free of all ectoderm. The extent of the distal development depended on the stage when the ectoderm was eliminated. Additional positive involvement of ectoderm in limb development was indicated by the work of Steiner (1928) with anura and Filatow (1928) and Balinsky (1931) with urodeles. These experiments are not cited more fully since they certainly lack the conclusiveness of those of the Harrison group.

COMPARATIVE DEVELOPMENT OF LIMB BUDS

Limb buds of amphibia are not structurally the same as those of other vertebrates. In most other vertebrates (mammals, birds, reptiles and even some fish) the early limb buds characteristically have a thickened ridge or crest of ectoderm along the free edge. This has been called the apical ectodermal ridge (Saunders, 1948). Most amphibia have no such structure at the free edge. At best there is a thicker cap of ectoderm, similar to the initial stages of a regeneration blastema, at the distal end of the limb. This ectodermal cap may have some importance for continued limb development, at least in anura, but this is frequently not evident because the cap can be regenerated fairly readily following its elimination (Steiner, 1928). The apical ridge of the chick limb bud is not replaced by regeneration (Saunders, 1948). These differences are basic and probably are responsible for a good many of the divergencies of limb development seen when the amphibia and birds are compared.

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The importance of the apical ectodermal ridge of the chick embryo's limb bud was shown by Saunders (1948). When the ridge was removed distal development of the limb ceased even though the mesoderm was not injured and the rest of the limb ectoderm quickly healed over the exposed area. The extent of distal development depended on the stage of the limb bud at the time of the operation. Even the ectoderm which was closely adjacent to the ridge could not form a new one. These observations were confirmed in a "wingless" mutant of chickens (Zwilling, 1949). The "wingless" condition was due to a recessive mutation which, in homozygotes, eliminated Saunders' ridge from the wingbuds at an early stage. Distal development of the wings failed, just as in Saunders' experiments.

There have been reasons to believe that Saunders' ridge was somehow involved in the development of other mutant conditions. Bretscher (1950) suspected this as a result of his experiments with polydactylous chickens. T. C. Carter (1954) went to the extent of calling the apical ridge "the limb inductor" when he elaborated an hypothesis to explain the polydactyly in luxate mouse mutants. It was obvious that a complete account of the relative importance of the two limb components, both for mutant conditions and in normal development, could best be obtained by some procedure which would allow for the interchange of ectoderm and mesoderm between limb buds. Such a procedure has been devised (Zwilling, 1955). The present communication is a synthesis of the results obtained from the application of this technique to the development of two mutant conditions as well as to several aspects of the development of genetically normal limbs. The information which has been obtained from the hereditary deviants has a central position in the elaboration of our hypothesis for limb development.

DEVELOPMENT OF LIMBS IN CHICK EMBRYOS

Normal chick embryo limb development has been described by Saunders (1948) and Hamburger and Hamilton (1951). In the earliest stages both leg and wing buds are fairly symmetrical. The ectoderm of the apical ridge at first is more columnar than adjacent ectoderm. Then it becomes similar to a pseudostratified layer of columnar epithelium and forms the typical nipple-like ridge or crest to which we have referred. At stage 17 the more columnar ectoderm of the developing ridge is fairly uniform in thickness along its entire length. This changes quite rapidly and by stages 18 and

19 the ridge is considerably higher (4–5 layers of nuclei) at the apex of the bud than at the anterior and posterior ends where it merges with the body ectoderm. By stage 20 this central thicker or higher region of the ectodermal ridge is very obvious. In addition the leg bud has become asymmetrical: the anterior portion tapers gradually and the posterior part of the bud is more solid and is cut off more abruptly. This foreshadows a posterior bulging of the developing limb. The apical ridge of ectoderm is highest in the region of the bulge and tapers down at both ends—more rapidly at the posterior and gradually at the anterior end. At stage 26 the wing and leg asymmetries are pronounced (Fig. 1). There is a distinct difference between the leg and wing (the former with a more pronounced posterior bulge in the paddle region, the latter with a more extensive anterior bulge) and a difference between the pre- and post-axial edges in each. Again we note that the thicker apical ectoderm is restricted to the region of new outgrowth (the paddle or presumptive digit region) and corresponds with the limb asymmetries. At this stage we note that the ectoderm along both the pre- and post-axial edges of the 'arm' and 'leg' regions can no longer be distinguished from the thin lateral ectoderm. Special emphasis has been deliberately placed on the asymmetries of the limbs and the associated asymmetries of Saunders' ridge during this description. The reasons for this will be made clear subsequently.

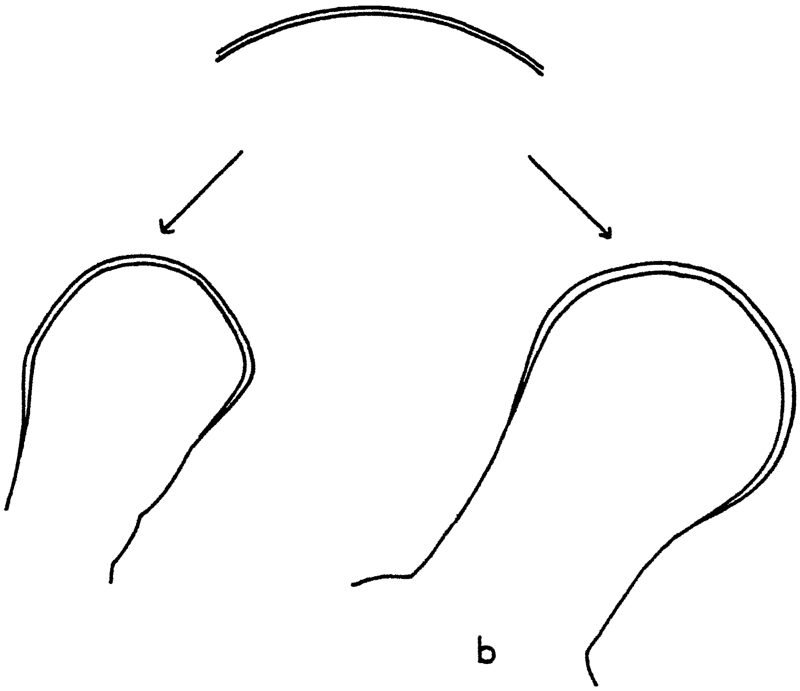

FIGURE 1. Diagram of generalized limb bud at early stage when the apical ectodermal ridge is uniform. This leads to the asymmetries seen in the stage 26 wing (a) and leg (b).

EXPERIMENTAL INTERCHANGE OF PARTS OF LIMBS

Experiments with the interchange procedure (mostly with stage 19 limb buds) have confirmed the importance of Saunders' ridge for limb development (Zwilling, 1955). Distal limb components do not form when the ridge is removed. Ectoderm from various other regions of the body has been placed over the limb mesoderm. The same has been done with ectoderm from younger embryos (5 to 16 somites). In a number of instances the latter ectoderm included the presumptive limb areas. Not a single case of development of distal structures has been obtained. In most cases only atypical girdle elements were found. Occasionally part of a femur or humerus formed. Lateral limb bud ectoderm (not including the ridge) seems to promote distal development somewhat more than ectoderm from other sites (unpublished). When such ectoderm was placed over the apex of a limb bud's mesoderm a fully formed femur or humerus developed. This conforms to Saunders' findings after he removed only the apical ridge. Again there were no cases in which more distal structures formed.

On the positive side we have found that distal limb elements developed whenever the apical ectodermal ridge was successfully fused to limb bud mesoderm. This was the case when the grafted ectoderm merely replaced the original in as nearly the same position as possible. It was also the situation when the apical ridge contacted other regions of the limb bud mesoderm. When the original apical ridge and about .1 to .15 mm of the underlying mesoderm were removed the remainder of the mesoderm could form an entire, fairly normal limb if it were provided with a new apical ectoderm from another limb bud. The strip which consisted of the original Saunders' ridge and underlying mesoderm also developed into a complete limb. Thus two limbs can form from one limb bud if an additional apical ridge is introduced into the system in an appropriate way. Various degrees of duplication of distal limb structures may be obtained if an ectodermal ridge (or part of one) is placed on each lateral surface of the intact mesoderm (deprived of all of its ectoderm) from one limb bud. We have had limbs, from such operations, in which there were only a couple of accessory digits. In others all structures distal to the humerus were duplicated (Zwilling, '56a). Thus, not only is the apical ectodermal ridge required for distal development of a limb, but it can promote or induce outgrowth and distal development from various parts of the limb bud.

EXPERIMENTS WITH LIMB BUDS FROM MUTANTS

Data which we have obtained from experiments with limb mutants indicate that the ectodermal ridge is dependent on the mesoderm for its continued persistence and activity. Mesoderm from a mutant limb was covered by genetically normal ectoderm. The reciprocal combination was also made and the limb buds from both combinations were raised as flank grafts on genetically normal hosts. When the mesoderm from genetically polydactylous buds was covered with normal ectoderm the polydactylous condition developed in the grafts (Zwilling and Hansborough, 1956). This was found to be true for limbs from two polydactylous mutations: wing buds from duplicate (Po^d) and leg buds from Silkie (Po) embryos. The pattern of the duplication in the grafts was almost identical with that of the control wing. Supplementary observations established that stage 19 duplicate wing buds did not differ in size or appearance from either genetically normal wing buds or phenotypic normals from the duplicate mating. A series of experiments were performed in which a piece of tantalum foil was inserted in a transverse incision through the center of the limb bud. This separated the limb buds into pre- and post-axial halves. When this was done to a normal wing bud only digit #1 developed from the pre-axial half. In the case of duplicate wing-buds digit #1 plus the accessory limb structures developed from the pre-axial half. At the time of the operation the pre-axial halves were identical in appearance in duplicate and normal wing buds. As the pre-axial half of the duplicate limbs developed, it was evident that the thicker more active region of the apical ridge was more extensive pre-axially than in controls. This anterior extension of the ridge was associated with the excessive pre-axial outgrowth and is noted consistently in the developing duplicate limbs.

That accessory limb outgrowth is dependent on the ectodermal ridge is emphasized by experiments in which only the pre-axial region of the ridge was removed from duplicate wing buds. If just the right amount of Saunders' ridge was cut off (Hansborough, unpublished) perfectly normal wings, minus any pre-axial duplication, developed. When all of this evidence is evaluated one has the following sequence of events: 1. at first neither the ectoderm nor mesoderm of polydactylous limb buds differ from the normal. 2. polydactylous limbs develop a greater pre-axial outgrowth than normal. This has associated with it a more extensive active apical ectodermal ridge which induces the excess outgrowth. 3. The same sort of extension of the apical ridge is developed in genetically normal ectoderm when it is in contact with polydactylous mesoderm and the typical accessory digits form. There is only one conclusion from these facts, namely that the extension of the thicker apical ectoderm of the ridge results from some influence present in the mutant mesoderm and absent in genetically normal limb mesoderm.

Similar combinations of mutant and genetically normal limb bud components have been made with wing buds from embryos homozygous for a wingless mutation (Zwilling, '56a). Wing buds appear at the usual time in such homozygotes. An apical ectoderm which is typical for stage 17 or 18 is present. But it never progresses beyond this stage, never becomes thicker. Instead it regresses and is completely absent by stage 23 or 24 (by the fourth day). Typically the only trace of the wing is the girdle —which develops reasonably well. Some of the homozygotes also have abnormal legs. However the apical ectodermal ridge persists for a longer time in the leg buds so that more of the leg develops. Frequently there is a normal thigh and the limb ends abruptly somewhere along the tibia, which is spike-like. Occasionally there are deficient digits. The cessation of distal development is linked to the time when the apical ridge regresses. When mesoderm from homozygous "wingless" wing buds was covered by genetically normal ectoderm and grafted to genetically normal hosts the results were quite consistent. In each case the wing developed more than did the control (the unoperated wing left in situ in the donor). But in each case the genetically normal apical ridge regressed sooner or later and distal development stopped. The limbs were spike-like structures with an abruptly terminated ulna. In one or two cases there was an attempt at the formation of the more distal elements but these were atypical. These graft wings were very similar to the legs in which the apical ectodermal ridge had persisted for a longer time than is usual for the wings.

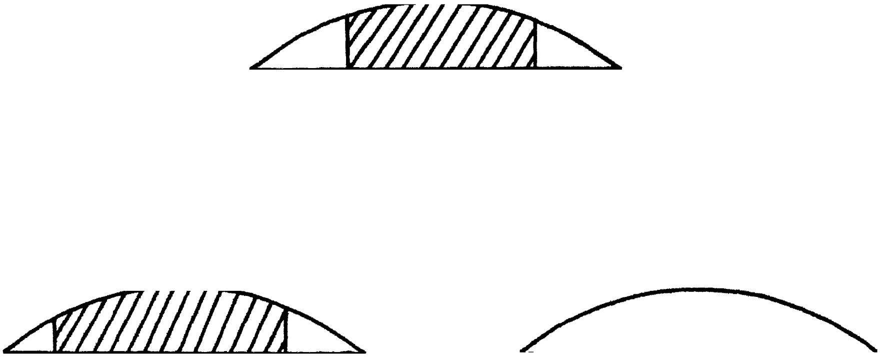

FIGURE 2. Diagram to show distribution of the ectodermal ridge maintenance factor in the mesoderm of a) normal limb bud, b) pre-axial polydactylous limb bud and c) wingless limb bud.

Evidently one of two things is happening in the wingless homozygotes. Either something detrimental to the continued existence of the apical ectoderm is produced by the limb mesoderm or something necessary for the continued existence of Saunders' ridge is missing. There is, at the present, no experimental basis for a choice between these alternatives. However, if one adopts the latter possibility one may have a reasonable hypothesis which accounts for all of the data so far obtained in experiments with limb buds. The hypothesis is this: outgrowth of the limb mesoderm and the formation of distal structures is dependent on some inductive stimulus which comes from the apical ectodermal ridge. The apical rdige is, however, not independent and self-sustaining. It depends upon some factor normally present in the mesoderm for its continued existence as an active (thick) structure. This can, at the present, be regarded merely as a maintenance factor. Moreover this maintenance factor for the apical ridge is not distributed uniformly through the limb bud mesoderm. More of it is present in the central region of the limb bud and less at both ends (Fig. 2a). If this were not so and if the apical ridge were equally active in stimulating outgrowth throughout its length then limbs would be round burgeoning structures. According to this hypothesis the results with both of our genetic deviants can be explained. Polydactylous conditions result when the maintenance factor is distributed farther preaxially than in normal limb buds (Fig. 2b). This produces a more extensive apical ridge and consequently more preaxial outgrowth. Winglessness is a consequence of the absence of the maintenance factor (Fig. 2c). The ectodermal ridge forms, but cannot persist because of this deficiency. Ergo—no outgrowth. When the maintenance factor is present in minimal amounts, as in the legs of homozygotes, distal development may continue as long as the factor lasts, but eventually the apical ridge regresses and distal outgrowth stops.

All of the limb asymmetries, which were described earlier, may be accounted for by an asymmetrical distribution of the maintenance factor. The regions of high activity of the ridge would then correspond with the regions of the mesoderm in which there are high concentrations of the maintenance factor. Supporting evidence for this view comes from some of our experiments with normal limb buds.

The combination of wing bud mesoderm and leg bud ectoderm developed into a perfect wing. The reciprocal combination yielded a normal leg (Zwilling, 1955). The apical thickening conformed to the pattern of distribution of the maintenance factor in the mesoderm. When ectoderm was rotated 180° before it was placed upon the mesoderm from an homologous bud the ectodermal ridge again conformed to the mesoderm and all of the axial relations of the limb which developed were those expected of the mesoderm (Zwilling, 1956c). In another experiment the thick, most active regions from two or three apical ectodermal ridges were placed in tandem along the apical edge of the mesoderm from one limb bud. These regulated so that the distribution of thin and thick ridge was normal in a short time and normal limbs developed (Zwilling, 1956a).

We may return now to the introduction and compare the chick limb buds with those of amphibia. Is the mesoderm of the chick limb bud "the positive factor of limb formation"? It contains the factor necessary for the existence of the ectodermal ridge and thereby also controls the pattern of development. These are certainly important positive attributes. But none of these traits can be expressed unless the proper kind of limb ectoderm is associated with the mesoderm. Unlike the situation in the amphibia, other ectoderm cannot, at the stages tested, take the place of the apical ectodermal ridge nor can the latter regenerate when it is completely excised. Therefore, it too, is an important "positive factor" for limb development. The nature of the relationship of the two "positive factors" for limb development is such that one cannot express its morphogenetic potentialities without the other—there is a strong reciprocal dependence between them. We have performed an experiment which indicates that the nature of this reciprocal relation may be altered and that the ectoderm may control one of the "positive" features usually resident in the mesoderm. As indicated above, when the ectoderm was rotated 180° the axial relationships which developed were those of the mesoderm. However, when the ectoderm was rotated 90° in similar experiments all of the axial relationships were determined by the ectoderm (Zwilling, 1956c). In assessing capacities of the components of systems such as this, one must be careful to evaluate them under conditions of greater and lesser stability. Unsuspected properties may be revealed when the system is unstabilized. Future experiments may show that the ectoderm is capable of controlling even more of the "positive factors" of limb development under other altered conditions.

SUMMARY

An hypothesis is presented which postulates that limb bud ectoderm and mesoderm have a reciprocal dependence. According to this view the chief function of the limb bud's apical ectodermal ridge is to promote continued outgrowth of the mesoderm. In addition to responding to this stimulus the mesoderm contains a factor for continued persistence of the ectodermal ridge. Asymmetries of the limb develop because of the asymmetrical distribution of the maintenance factor in the mesoderm. This determines which regions of the ectodermal ridge will become thick and active and thereby controls many aspects of limb pattern. The action of two mutations which have been studied seems to be primarily upon the maintenance factor. The distribution is altered in polydactylous limbs while the wing buds of a "wingless" mutant appear to be deficient in the maintenance factor.

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LETTERS TO THE EDITORS

Correspondents alone are responsible for statements and opinions expressed. Letters are dated when received in the editorial office.

A DESCRIPTION OF A RED DRUM, *SCIAENOPS OCELLATA*, WITHOUT EYES, WITH A CONSIDERATION OF THE IMPLICATIONS

The red drum or redfish, as it is called on the Gulf Coast of the United States, is possibly more prone to the development of certain anatomical abnormalities than other fishes. I described two specimens from Texas with reversed scales (Gunter, 1941, 1945a). I also listed a specimen without eyes (Gunter, 1945b), on the basis of a report of a commercial fisherman. This faith has been verified finally by the acquisition of a second specimen without eyes.

The specimen was caught in Alazan Bay on the south Texas coast some two to four days before it was brought to Mr. H. C. Ware, of Riviera, Texas, on June 21, 1955. The collector and the method of capture are unknown to me. Mr. Ware gave the fish to the Marine Biology Laboratory of the Texas Game and Fish Commission at Rockport, and I am deeply indebted to Mr. Howard Lee and Mr. Cecil Reid of that laboratory, who, knowing my interest in the specimen, generously presented it to me.

The fish was 63.5 cm. (25 in.) in fork length and 68.6 cm. in total length. It had been gutted and gilled and weighed seven pounds in that state. It was a male and the testes were fairly well-developed. The animal would have spawned in the fall. It was in good condition and there was a considerable amount of fat on it. After the preliminary examinations and the photographs were made, the body was eaten because it appeared to be perfectly normal. The head was preserved and is in my possession. Anatomical studies will be made later in comparison with a normal redfish, and the results will be presented elsewhere.

Plate I shows that the fish had no eyes at all and not even vestigal eyes are apparent from the external examination. It is doubtful that the fish was light-sensitive.

DISCUSSION

The redfish belongs to the Sciaenidae or drum family, which is in many ways the most successful family of fishes on the northern Gulf coast (see Gunter, 1938). It is a fast, strong, active fish, highly valued by both sportsmen and commercial fishermen alike. It is a predatory species feeding largely on shrimp and crabs and is not a bottom feeder like its relative, the black drum, *Pogonias cromis*, which subsists largely on molluscs.

PLATE I-1a. A head view of the eyeless redfish.

The black drum has sensory barbels under the chin, the redfish does not. Yet the redfish can change its habits to such an extent that it can exist perfectly well, so far as bodily condition indicates, in a completely blind condition.

Mr. Samuel Gampert, a long-time fisherman at Port Aransas, Texas, told me that he once caught a blind redfish in a cast-net. It had opaque eyes and presumably had come from the Laguna Madre, where the high salinity sometimes causes fishes to go blind. He released it and it moved away, not with the headlong rush of a normal redfish under such circumstances, but very slowly.

The first blind redfish reported (Gunter, 1945b) was sold on the open market and it must have been in good condition. The flesh of the specimen reported here was in excellent condition. Blind redfish must have different feeding habits from normal individuals, but how different is not known, and they may, conceivably, not be greatly different for redfish sometimes feed on the darkest nights when nothing can be seen.

Plate I-1b. A view of the left side of the whole fish.

Redfish with intermediate development of the eyes are not known. A few may be hatched without eyes, but unfortunately there is no surety about that question. Pew (1955), who gave a popular account of this specimen, has pointed out that the eyeless condition could be the result of a congenital anomaly, a pathological anomaly, heredity, or physical removal of the eyes by parasites or predators. It should also be noted that the specimen came out of the Laguna Madre, where the hypersaline condition sometimes causes redfish and other fishes to go blind. Possibly the high salinity could cause the eyes of a small redfish to degenerate. Unfortunately, it is doubtful that the question can be answered by anatomical dissection.

It is generally assumed that the evolutionary degeneration of the eyes of certain cave animals is slow. The existence of successful blind redfish indicates that this is not necessarily true, at least from the ecological standpoint.

If such eyelessness in redfish is hereditary, then in the cases reported, in time and space, two "hopeful monsters" almost met. The same can be said of the two fish with reversed scales. However, it is scarcely conceivable that such progeny could compete successfully with their better endowed congeners.

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MELANOTIC TUMORS IN DROSOPHILA AS POSSIBLE PHENODEVIANTS

Several investigators of the so-called benign melanotic "tumors" in Drosophila (Burdette, 1954; Wilson, King and Lowry, 1955, and others) have established that the appearance of these tumors is genetically conditioned. Such tumors appear spontaneously in scattered individuals in most laboratory stocks of *Drosophila melanogaster;* they have also been found in wild flies of several Drosophila species collected in nature, and in the progenies of such flies in strains maintained in laboratories (Brncic and Koref, 1953). The frequencies of spontaneous tumors are generally low, of the order of 1 per cent or lower. Makino and Kanehisa (1951) showed however, that these frequencies can be substantially increased by inbreeding, although seldom to 100 per cent. The morphology, localization and number of the tumors in the body of the larva or adult fly are characteristic for different stocks.

The genetic studies of Burdette and other authors have been interpreted to mean that the tumors are produced by one, two, or more specific genes in different stocks. These genes are always incompletely penetrant, and vary in expressivity, sometimes due to systems of modifiers. The main genes and modifiers responsible for the production of tumors have been shown to be localized in all chromosomes, except the small fourth in *Drosophila melanogaster.* However, these genes in different strains are, with one known exception, not allelic (Burdette and Olivier, 1952). The increase of tumors is likewise greatly influenced by environmental variations. (Burdette, 1954; Wilson, King and Lowry, 1955; Brncic and Koref, 1951, 1953).

Our recent studies lead us to believe that the appearance of melanotic tumors in Drosophila may best be interpreted in the light of the recent work of Lerner (1954) and others. These tumors may be what Lerner has called "phenodeviants." The incidence of tumors seems to depend not so much on individual mutant genes as on the general genetic environment, the residual genotype, of the strains in which they are found. Table 1 summarizes some relevant data. Three strains of *Drosophila melanogaster:* tu^{48j}; tu^{49h}, and tu vg bw, have been used. The incidence of adult flies in these strains showing one or more melanotic tumors is 48.3, 63.2, and 99.9 per cent, respectively. Each of these strains was outcrossed to the wild Oregon R-c strain and to seven different mutant strains listed in the table. In the F_1 generation of these crosses no tumors at all have been found in the 200 flies examined from each cross (a total of 4800 flies). The tumors have reappeared, however, in the F_2 hybrids. The table shows that the frequencies of the affected flies were very different in different crosses, although always lower than in the original tumor stocks. The crosses to the Oregon R-c strain have been taken as the standard; it is known that tumors in this strain are rare (about 0.1 to 0.2 per cent). The column marked "Sig. Dif."

in the table shows the differences between the cross to Oregon R-c and to other strains, divided by the standard errors of these differences. In several cases the ratios are higher than three, and therefore indicate statistically significant deviations.

TABLE I

INCIDENCE (IN PER CENT) OF TUMORS FOUND IN F2 IN CROSSES BETWEEN TUMORAL AND NON TUMORAL STOCKS.

Non tumoral stocks	tu 48j			tu 49h			tu vg bw		
	No. of indiv.	Per cent of tu	sig. Dif.	No. of indiv.	Per cent of tu	Sig. Dif.	No. of indiv.	Per cent of tu	Sig. Dif.
Oregon R-c	2085	0.48	...	2122	2.81	...	2254	3.54	...
w	2034	0.35	0.65	1667	2.84	0.05	2213	2.45	2.01
f	1566	0.32	0.77	1314	2.89	0.13	1914	1.00	5.64
dp	2116	1.4	3.2	1760	3.53	1.28	1667	16.73	13.32
bw	2016	0.50	0.09	2262	8.44	8.13	2179	5.26	2.81
st	2076	0.48	0.00	2196	5.07	3.81	2119	2.81	1.004
se	714	8.02	7.39	2750	29.17	28.34	1838	12.13	10.105
ey	2042	0.54	0.02	2081	3.02	0.41	2198	4.86	2.2

We may, then, conclude that: (1) Tumors appear in most species and strains of Drosophila. (2) They are produced by different "principal genes" in different strains, most of them non-allelic. (3) The penetrance is characteristic for each strain, and depends on the residual genotype of this strain. (4) The frequency is increased by inbreeding and decreased by outbreeding. (5) The frequency is sensitive to environmental influences. All these characteristics of the "tumors" resemble those of traits such as "podoptera" in Drosophila (Goldschmidt, Hannah and Piternick, 1951), and as "crooked toes" in chickens (Lerner, 1954). These traits are considered by Lerner as "abnormal morphological deviants (phenodeviants) which occur sporadically, caused by the intrinsic properties of Mendelian inheritance, due to which a certain percentage of individuals of every generation falls below the threshold of the obligate proportion of loci needed in heterozygous state to insure normal development."

It may be interesting to consider the possibility that susceptibility for the production of tumors in Drosophila, and perhaps in other animals, may not be caused by mutations at specific loci. Their origin may rather be due to loss of the normal genetic equilibrium, or of genetic homeostasis in certain strains. This loss may be produced in particular by inbreeding, connected or unconnected with selection. If so, the production of tumors is due not to genes specifically concerned with tumors, but to alleles of loci which exist in most normal individuals of a species or a population.

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THE DIFFERENTIATION OF TISSUE CELLS[1]

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INTRODUCTION

One of the significant achievements of the experimental analysis of metazoan development is the concept of determination—that during embryonic differentiation the various organ and tissue primordia pass through stages of increasing specificity and stability until they finally form characteristic tissue types. Various experimental methods have demonstrated this increasing restriction of potencies to be a typical feature of the development of all metazoa, though the pace at which it is manifested may vary from group to group. Because of the stability generally demonstrated for tissues at advanced stages of differentiation, the conclusion has been drawn that the various tissues, *and their constituent cells*, are at this time irreversibly fixed. According to this point of view, once cells have differentiated into the characteristic histological types, fundamental changes in cell type are no longer possible (for example, thyroid epithelium to cartilage). Any changes in cell morphology or behavior that may be observed are considered to be minor, reversible changes within a cell type. Thus all modifications in cell form that occur during development may be fitted into one of two categories (Weiss, 1939, 1953). If they are stable and persist in the absence of the initiating stimulus they are considered to be "true differentiations" (irreversibly fixed). If on the contrary they are unstable, that is, do not persist upon removal of the initiating stimulus, they are termed "modulations."

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[1] An expansion of a paper read at the symposium on *Problems in Differentiation* before the American Society of Naturalists, East Lansing, Mich., September 8, 1955.

[2] My analysis of this problem has been greatly stimulated by discussions with a number of my colleagues; in this respect I am particularly grateful to Doctors Edgar Zwilling and Clifford Grobstein.

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These definitions have brought order into our thinking and have focused attention on differentiation at the cellular level. They have therefore materially influenced our approach to the problem of differentiation. The view that not all changes of cell character are of similar stability or of equal developmental significance has in particular had a salutary effect on discussions of this problem as in the interpretation of the morphological dedifferentiation that is routinely observed in tissue cultures (Champy, 1912) and in the formation of the regeneration blastema.

Although there have been voices urging caution (such as Bloom, 1937); Ephrussi, 1953; J. Needham, 1942; Spiegelman, 1948) and voices of protest (especially Harrison, 1933), the principle of irreversible differentiation has found widespread acceptance. It has in some instances even served as an established fact of development, upon which theorizing on the mechanism of cellular differentiation could be based (Darlington, 1948; Fischer, 1946; Lillie, 1929; Medawar, 1947; Monod, 1947; Powell, 1946; Rusch, 1954; Weiss, 1939, p. 468, 1950, 1953; but see Weiss and James, 1955). Sonneborn (1947) formerly belonged to this group but has since taken a more circumspect position (1949).

With any topic of such fundamental significance constant reexamination is necessary. It is the purpose of this paper to consider the present state of our knowledge of tissue cell stability. To this end we shall reevaluate the evidence upon which our thinking has been founded, ponder the significance of recent discoveries, and suggest general approaches for future investigation.

CRITIQUE OF THE EVIDENCE

Certain difficulties in the determination concept were emphasized some years ago by Harrison (1933) in his famous treatise on this problem. In tests of the fixity of a primordium one frequently obtains different answers with different methods. In such instances it is proper to ask whether the causes of limited differentiation reside solely in the tissues, or in the imposed environment as well. Harrison further warned that the determination concept, resting as it does on negative evidence, is necessarily lacking in scientific certitude. "There is no way of finding out with certainty whether the particular quality which a cell seems to have is finally fixed, for there always may be new conditions, not yet tested, under which other potencies may be revealed." (1933, p. 318; see also Ephrussi, 1953, p. 99). Certainly in this, a fundamental problem of development, there is need for careful specification of the conditions of our experiments; and before generalizations can be accepted, plausibility must be fortified by rigorous examination of the results.

1. *The Criterion of Self-Differentiation*

a) *The Evidence from Tissue Culture.* The presumed fixity of tissue cells rests almost entirely on the criterion of self-differentiation—the capacity of the part in question to carry through or preserve its type-specific differentiation when isolated or explanted in a so-called neutral environment in vitro. [For Lillie (1929) this is the only acceptable criterion.] In most discussions of the fixity of differentiated tissue cells the demonstrated stability of certain tissues in long term tissue culture looms as the principal evidence (Ephrussi, 1953; Fischer, 1946; Lillie, 1929; Monod, 1947; Rhoades, 1943; Sonneborn, 1949; Weiss, 1939, 1944, 1949, 1950; Wilson, 1925, and others). It is necessary, therefore, to examine this work in some detail.

Although there have been many claims that differentiated tissues preserve their specific character in long term tissue culture, not all of these have been substantiated (cf. Bloom, 1937; Willmer, 1954). We will therefore concern ourselves only with cases whose validity is accepted, and which in turn have been most widely quoted.

Parker (1929) discovered that even though a tissue loses its distinctive histological character under optimal growth conditions in culture, it nevertheless betrays its source by its rate of outgrowth in standard chicken plasma-embryo-extract medium. Thus fibroblast cultures derived from periosteal tissue have a higher rate of outgrowth than those from embryonic heart. Furthermore, so-called pure cultures of these fibroblasts preserved their distinctive rates of outgrowth in vitro for 97 days (Parker, 1933). When heart fibroblasts were then cultured in a medium rich in embryo-extract, and periosteal fibroblasts were cultured in a medium poor in embryo-extract, the relative rates of outgrowth were reversed. But upon return to the original regimen, the tissues reverted to their original rates of outgrowth!

It seems indubitable that under stable growth conditions in culture these cell strains possess a degree of stability. But these results do not justify Parker's (1932) conclusion that "The organism is able to effect progressive and irreversible changes in the constituent cells." Leaving aside the questionable significance of small rate differences in so complex a process as outgrowth, let us re-examine the conditions of the experiment. Cells from a single tissue or organ are cultured in an isolated state in a standardized medium in which nutrient materials are derived from the entire embryo. If one wished to design an experiment which would protect a tissue from other histogenetic stimuli and preserve, as far as possible, the specific character of the cells, it would be difficult to improve on this model. Is it therefore surprising that under such conditions certain characteristics of a tissue are stable?

A further indication that this stability may be spurious stems from the observation that the activity of fibroblasts in culture varies with the age of the embryo; skeletal muscle fibroblasts from 17-day chick embryos have a higher rate of outgrowth in culture than those from 8-day embryos (Parker, 1932). Apparently under *in vivo* conditions there is a change in character of this tissue between the 8th and 17th days of development. However, there is no such change *in vitro*. Fibroblasts from 8- and 17-day skeletal muscle are found to preserve the rate of outgrowth possessed at the time of explantation for as long as 104 days under standard culture conditions (Parker, 1932). Maintenance of tissue character in these studies seems to be due more to the stability and generalized character of the medium than to the stability of the tissue cells. Under these circumstances the tissue cells appear to be in a state of developmental arrest.

Other oft-quoted examples involve the restoration in culture of the characteristic differentiation of certain tissue cells after its loss under optimal growth conditions. Pigmented iris epithelium depigments during 8-10 passages in media rich in embryo-extract, but reforms pigment granules under poor growth conditions with removal of the embryo-extract (Doljanski, 1930). When anterior hypophysis is cultured at 37 degrees in a medium rich in growth factors, no eosinophil or basophil cells are formed; but when nutrients are reduced and the culture kept at a lower temperature (28°-30°), many cells appear with abundant eosin-staining granules (Kasahara, 1935). Similarly, sheet outgrowth of kidney epithelium redifferentiates into tubules, when a fragment of connective tissue is added to the culture (Drew, 1923).

In these and like studies a certain stability of the tissues under the imposed conditions is indicated. Certainly a partial redifferentiation of iris epithelium and hypophysis is clearly demonstrated. This is quite to be expected, however, inasmuch as it occurs under generalized conditions. Reducing the mitotic rate and particularly the cell movements merely creates conditions that, being more like those *in vivo,* encourage differentiation. In the absence of new influences the tissues apparently follow the path of least resistance, and redifferentiate according to type. It may be noted, incidentally, that even under these generalized conditions tissues do not always redifferentiate. If iris epithelium is cultured in such a manner that it becomes deficient in nutrients and oxygen, it may form keratin-like material (Fischer, 1924).

The formation of tubules by kidney epithelium when combined with connective tissue is less clear. This may not be a redifferentiation of kidney tubules at all, but rather a non-specific reaction of an epithelium to a change in its tissue associations (cf. Levi, 1934; Bloom, 1937). Iris epithelium, for instance, will form duct-like structures when combined in culture with heart fibroblasts (Ebeling and Fischer, 1922; Fischer, 1946, p. 225).

We must then conclude that while many studies of self-differentiation in tissue culture definitely demonstrate stability under the imposed conditions, these studies do not by any means provide final evidence for irreversible fixity of tissue cells. On the contrary, they present evidence that different in vitro environments may elicit different responses from cells. Stability of cell type in culture appears to depend upon stability of the environment.

b) *The Significance of Mass.* Our conclusions on cell potency have been based almost entirely on experiments involving tissue masses consisting of many cells. A fallacy in these conclusions is revealed by the repeated observation that the degree and variety of differentiation obtained tends to vary directly with the mass of tissue involved (Andres, 1953; Berrill, 1945; Chalkley, 1945; Child, 1928; Galtsoff, 1925; Grobstein, 1952; Grobstein and Zwilling, 1953; Lopaschov, 1935). Thus explants of presumptive head mesoderm from a single early gastrula of *Triton taeniatus* form only muscle *in vitro,* but larger masses, consisting of identical cells derived from the fusion of several of these same primordia, form muscle, chorda, brain, sensory primordia and ectoderm (Lopaschov, 1935). In the reconstitution of masses of minced Hydra the number of hypostomes, feet, tentacles and cavities formed increases directly with increase in the mass of tissue (Chalkley, 1945). Apparently, with increase in number of cells, along with tissue mass, cell movements and interactions increase in complexity until at certain levels new capacities of cells emerge. Hence, a description of the potencies of a given tissue mass does not necessarily reveal the capacities of the individual constituent cells. This raises the possibility that when a tissue mass is judged to be determined by the usual criteria (for example, self-differentiation in explants and transplants) individual cells within the mass may retain wider potencies. It seems possible that in transferring a mass of cells to a new locus the immediate environment of the inner cells is but slightly altered. Such a slight change may be sufficient to effect a shift in cell differentiation during the earliest phases of development (as in early amphibian gastrula); but for later stages, generally considered to be fixed, a more acute change may be required. In such instances fixity of the mass could not be taken as evidence of cell fixity, and the status of cytodetermination would remain open.

c) *The Differentiation of Isolated Blastomeres.* Much thinking on the irreversibility of the determination process stems from studies on isolated blastomeres of eggs with so-called mosaic development (such as annelids, molluscs, tunicates). These studies, for example, form the main basis for Lillie's (1929) theory of embryonic segregation. The fact that the development of isolated blastomeres *in vitro* does not exceed their fate in normal development has been generally accepted as proof that an extraordinary degree of fixity is achieved before and during early cleavage in these eggs (Wilson, 1904 a, b; Conklin, 1933; Costello, 1945). That there is remarkable stability under the imposed conditions cannot be disputed; but again we may ask: is this due primarily to the state of the blastomeres or to the method of culture? The observation that in many instances blastomeres actually undergo less segmentation and differentiate more poorly in isolation than they do in the intact embryo gives weight to the suggestion (Costello, 1945, p. 53) that the culture conditions are not optimal for differentiation.

In all of these studies individual blastomeres are cultured in a relatively large volume of non-nutrient medium (approximately 0.5 cubic centimeter or more of sea water) in contact with air. Modern tissue culture methods would suggest several improvements in this procedure. It now seems well established (Earle, Bryant, and Schilling, 1954) that a medium conditioned by unknown products of living cells is essential for the proliferation of single tissue cells or small clusters of cells in culture. This may be achieved by drastic restriction of the volume of the medium, so that the cells remain in association with their own metabolic products (Sanford, Earle, and Likely, 1948), or, more effectively, by the use of x-irradiated "feeder" cells (Puck and Marcus, 1955). Child (1928), Grobstein (1952), Grobstein and Zwilling (1953) and others have shown that the mass of a given primordium must exceed a given level if complex histogenesis is to occur in culture. A further factor influencing the differentiation of tissues in culture is the CO_2 tension in the medium (Spratt, 1949; Trinkaus and Drake, 1956). This suggests that the beneficial effects of metabolically conditioned media (see above) may be partly due to a rise in CO_2.

It seems likely that if these lessons were applied to the study of isolated blastomeres, more complete development would result. If the volume of the medium were greatly reduced (or several blastomeres cultured together), and the CO_2 tension raised, or if conditioned medium were provided by the method of Puck and Marcus (1955), increase in cellular proliferation may follow. By these means alone a state may be attained in which more complete differentiation is possible. We may also increase the degree and variety of differentiation by increasing the mass of a given type of protoplasm (cf. Lopaschov, 1935), perhaps by the aggregation of several blastomeres of one type, for example, several $1a^2$ micromeres. In view of the fact that the *in vitro* environment of an isolated blastomere in these experiments (sea water) is markedly different from its *in vivo* surroundings (other cells, intercellular material, colloidal material of the perivitelline space), attempts at nutritional enrichment of the medium are in order. This might prolong viability (generally low in these experiments), and thus increase the possibilities of differentiation (cf. Penners, 1937). Finally, following the lead of Yamada (1950) and Gallera (1953) (see below), chemical shock treatment of blastomeres may prove fruitful. This may disengage protoplasmic organization sufficiently so that when subsequently cultured other potencies may be revealed.

In summary, it seems possible that application of our improved knowledge of the requirements of tissues for proliferation and differentiation in culture to the classical problem of the mosaic egg may elicit different results. Until this is done our knowledge of the potencies of individual blastomeres of these eggs must be considered incomplete.

II. *Cellular Transformations*

Thus far it has been our concern to demonstrate the inadequacy of the evidence upon which belief in the fixity of tissue cells has been based. Cogent though these objections may be, the most convincing evidence against cell fixity would be a positive demonstration of the transformation of characteristic, cytologically differentiated cells into distinctly different cell types (metaplasia).

There are now a few studies in which just this has been shown to occur. It has been well-established for some years that upon removal of the lens from the eye of larval and adult newts a new lens may differentiate from pigmented marginal cells of the dorsal iris (for references see Reyer, 1954). Similarly, in Triturus, Stone (1950) has elegantly described the step by step transformation of retinal pigment cells into characteristic neural retina, complete with rods and cones. And now quite recently, Fell and Mellanby (1953) have demonstrated that in the presence of high levels of vitamin A body-wall skin of 6–7-day chick embryos will form ciliated, mucous-secreting epithelium in culture (see also Hopper and Mathews, 1953). This transformation is of particular interest. It suggests that vitamin A may be as important in the embryonic histogenesis of mucous membranes as it is in the maintenance of these tissues in the adult, and, as a defined chemical effect occurring in vitro, it is amenable to quantitative analysis. As a first step, Weiss and James (1955) have indicated that vitamin A has primarily an inducing rather than a supporting role, the induction occurring within a period of 15–60 minutes. This finding has considerable general significance, for it raises the possibility that the mechanism in this transformation, and in others as yet unanalyzed, is similar to that proposed a number of years ago for certain types of chemical carcinogenesis, where the combined necessity of "initiating action" and "promoting action" has been emphasized (cf. Berenblum, 1954).

Changes in cell type in the dorsal iris, the pigment layer of the retina, and in 6–7-day chick ectoderm are spectacular indications of pluripotency in tissues in advanced stages of differentiation, and as such administer a telling blow to notions of tissue cell fixity. In assessing their general significance, however, it must be established whether these are specialized, aberrant occurrences, or the first discovered examples of a widespread phenomenon. Certainly we can find no justification at this point for assigning the lability found in these particular cells to other types of tissue cells; but these studies nevertheless encourage us to raise the question of transformation of cell type as a possibility for all types of cells in all stages of differentiation.

In this context we have reason to focus our attention briefly on other instances of cell transformation. Although these involve cells in early stages of differentiation and are therefore less convincing for the argument, they serve to emphasize the possible general nature of the process. These studies are also of considerable methodological interest, employing defined chemical means to shift the course of differentiation. By exposing ventral marginal zone of early amphibian gastrulae to ammonia solution at pH 11.8–12.2 Yamada (1950) caused this material to form notochord, muscle and nephric tubules in culture. Controls, lacking the shock-treatment with high pH, formed blood islands, blood vessels, mesothelium and nephric tubules. An ingenious method of pretreating amphibian neurulae with urea solution enabled Gallera (1953) to increase the competence of its otherwise unresponsive ventral ectoderm to neural induction, so that when grafted to the neural plate area it formed brain, optic vesicle, etc. A further sign of lability in the ventral ectodem of the neurula is found in Wilde's (1955) demonstration that in vitro treatment of this layer with a presumed melanin precursor, phenylalanine, elicits the differentiation of characteristic dendritic pigment cells, like those of neural crest origin.

In addition to the aforementioned, in which changes in cell type are definitely demonstrated, there are a number of other studies in which the interpretation is not yet clear. Be that as it may; since they are suggestive of the possible general lability of tissue cells, they merit consideration. It was found some years ago that bone and cartilage formation may be initiated in the skeletal muscle of adult rabbits by implantation of periosteal tissue and alcohol extracts of bone (Levander, 1938). Although it has since been established that this is not a specific induction but rather a non-specific reaction to irritating substances (Heinen, et al., 1949), cellular transformation is certainly involved. The question is whether the bone or cartilage originates from fully differentiated tissue cells, such as muscle or connective tissue cells, or from more generalized, pluripotent cells.

The tendency of fibroblasts ("mechanocytes"—Willmer, 1945) to transform into macrophages (histiocytes) has been emphasized by a number of workers (cf. Bloom, 1937; Willmer, 1954). In some instances it hasn't yet been established whether the increase in number of macrophages is due to a cellular transformation or to a selective augmentation of the existing macrophage population (Chevremont, 1945, 1948). But even when morphological transformation to macrophage-type cells is observed directly (Weiss, 1944; Weiss and Wang, 1945), the significance of the change in cell form remains in doubt. Because of its generalized morphology, its ubiquity in the organism, and the relative ease with which other cells appear to assume its form, the macrophage presents an unusually difficult problem in histogenetic classification. We do not know whether to consider it a fully differentiated tissue cell, a morphologically generalized form of many types of tissue cells, or a histogenetically generalized, pluripotent cell. Depending upon which designation fits best, transformation of a particular type of cell into a macrophage would be, respectively, a change in specific cell type, a modulation, or a dedifferentiation.

The possibility that cell transformations play a role in pigment spread in spotted guinea-pig skin has attracted much attention of late (Billingham and Medawar, 1948, 1950), coming at a time when there is considerable interest in the role of plasmagenes in differentiation. These authors contend that pigment spreads from dark into white areas by the infective transformation of non-pigmented dendritic cells in the basal layers of the epidermis, rather than by pigment cell migration. In support of this hypothesis they have marshalled an impressive array of evidence. The argument has been greatly weakened, however, by the discovery (Billingham and Medawar, 1953) that the so-called white dendritic "cells" are in reality not located in the basal layers of the epidermis, as are the melanocytes, but in more superficial layers. Nor has there been any attempt to test the hypothesis by depriving skin of non-pigmented dendritic cells (cf. Rawles, 1944). If the infective transformation hypothesis is correct this should prevent pigment spread. In view of these objections (see also Lederberg, 1952; Rawles, 1955) the mechanism of pigment spread in this material remains unknown. An interesting variation on the theme of infective transformation has been proposed by Niu and Twitty (1950) for the origin of post-metamorphic skin melanophores in Triturus. They suggest that these cells are derived from "macrophages," which in turn have acquired their pigment phagocytically from cytolyzing melanophores. This is an intriguing possibility. However, in view of the possibility that the trypan blue stained cells in their material are really melanoblasts of neural crest origin and the fact that no branched melanophore was observed to have acquired its pigment by ingestion, we must reserve judgement.

Although the problem of cell origin in regeneration and carcinogenesis is a primary stimulus for continuing interest in the potencies of tissue cells, more than the briefest consideration of these phenomena would be beyond the scope of this paper. The concept of irreversible differentiation requires that the regenerate originate either by type-specific redifferentiation of tissue cells, such as muscle from muscle cells or bone from osteoblasts, or from relatively undifferentiated, pluripotent cells called *neoblasts*, or from both of these sources. The occurrence of regeneration in forms with determinate, mosaic cleavage (cf. Harrison, 1933), and a wealth of provocative experimental work supporting the possibility that single tissues are the cellular source of the regenerate (summary in Needham, 1952) seriously shake the foundations of the redifferentiation and neoblast theories. Nevertheless, due largely to the extreme difficulty of devising critical experiments, the problem of cell origin in regeneration remains open. In carcinogenesis, the concept of irreversible differentiation requires that the cancer originate either from neoblasts, or by modulation. The independent stability possessed by many tumors upon repeated transplantation (for example, Jensen rat sarcoma) indicates that many cancers are not modulations. The results of extensive investigations of chemical carcinogenesis certainly weaken the neoblast theory; but they do not invalidate it. The neoblast is an elusive entity and not readily eliminated. As long as critical information on cell origin is lacking final judgement must be held in abeyance.

III. *General Difficulties*

Finally, there are two additional difficulties in the concept of irreversible differentiation which, while not critical, call into question its usefulness as a research guide. In the first place, widespread acceptance of this concept has tended to discourage research on the potencies of tissue cells in advanced stages of differentiation. For if these cells are considered to be fixed, transformations of cell type are of course impossible, and all changes that occur must be modulations (even though the test of reversibility is often not applied). Secondly, it has led to confusion in assessing the significance of cell changes in histogenesis. According to this point of view, most of the characteristic cytological and histological characters of the organism are mere modulations; they disappear when tissues are isolated and cultured under optimal growth conditions. This results in a tendency to consider striking differences in cell morphology and behavior *in vivo,* such as bone versus kidney, to be less important than slight and questionable differences *in vitro,* such as rate of outgrowth.

CONCLUSIONS

It is clear that the evidence upon which the concept of irreversible differentiation has been based is inconclusive. The question of cytodifferentiation therefore remains open. Because of the incomplete nature of the evidence I take a frankly phenomenological position and define differentiation on the cellular level simply as an increase in the structural and functional specialization of cells. We cannot at present take a general position on the matter of cell stability.

This definition, although tentative and lacking in precision, has the advantage of reflecting the limited state of our knowledge of tissue cell stability. By implication there are several consequences of this. There is obviously a need for further research on the problem of cell transformation. In pursuing this subject, however, we have no reason to expect that all fully differentiated cells share a particular degree of stability. Different cell types may in fact vary widely, from extreme lability to complete fixity. It is conceivable, for example, that certain cell types may transform into almost any other type. For others, the instability may be limited to shifts in type within a histogenetic family (such as epithelia, nervous tissue, or mesenchyme and connective tissue). Some credence is given to this possibility by the occurrence of demonstrated and suspected transformations within such tissue families (Fell and Mellanby, 1953; Levander, 1938) and by the impressive evidence for nuclear differentiation recently presented by King and Briggs (1955). For still other cell types differentiation may be a truly unidirectional, terminal process, with only one type of end product possible. Perhaps this is true of presumed post-mitotic cells, such as neurones (but see Murray and Stout, 1947), erythrocytes, and neutrophils, and of certain tumor cell strains, such as Jensen's rat sarcoma.

In considering the mechanism of cell "transformation" we may be reminded that there are no compelling reasons for assuming that change of cell type must necessarily involve reversion to a more primitive stage in the normal differentiation of that type. Entirely new paths of differentiation may be followed. Another question of fundamental relevance to the mechanism of transformation concerns the role of cell division. Is cell division a prerequisite of transformation in any given instance? And finally, if lability is definitely indicated for a particular strain of cells, what are the control mechanisms that maintain these cells in a particular differentiated state during normal development and functioning? In brief, the potencies of tissue cells, mechanisms for the transformation of cells, and mechanisms for the maintenance of cell stability must be worked out in each instance.

Cytodifferentiation must eventually find its explanation in terms of an interaction between intracellular and extracellular factors. Hence, speculations on the role of nucleus, cytoplasm, plasmagenes, induced enzymes, and the like in cellular differentiation usually find a receptive audience. It must be recognized, however, that our present ignorance of the stability of tissue cells limits the validity of many of these theories, especially those which assume the inexorable fixity of tissue cells. [The discussions of Spiegelman (1948) and Ephrussi (1953) are provocative exceptions in this regard.]

PROSPECTUS

An attack on the problem of tissue cell stability must be founded upon the realization that apparent stability of these cells may not be due to their intrinsic properties but to properties of the tissue system as a whole. This may involve not only cell number and density, but the properties of the intercellular matrix (Grobstein, 1955). Consequently, in order to study the potencies of tissue cells they must first be released from their tissue system. There have been various attempts to achieve this by mechanical dissociation of the entire organism into cells and clusters of cells. (Brøndsted, 1936; Child, 1928; Galtsoff, 1925; H. V. Wilson, 1907; Weiss and Andres, 1952). These investigations have been useful in pointing the way, but the incompleteness of the dissociations, the extensive cell destruction, and the complexity of the cell suspensions have rendered the results difficult to interpret. The possibility of a more effective attack on this problem has emerged in recent years with the introduction of more subtle, chemical methods of dissociation. Holtfreter (1944) pioneered in this by using high pH to dissociate cells of early amphibian embryos. But this treatment seems to be ineffective with more advanced stages. The need for such an agent has been filled by the discovery that both trypsin (Moscona, 1952) and versene, ethylenediamine tetra acetic acid, (Zwilling, 1954) will cause many advanced primordia and tissues to dissociate into a suspension of individual cells possessing a high degree of viability. The use of trypsin as a dissociating agent has been discussed critically by Rinaldini (1954) and Harris (1955).

Once cells have been released from their tissue system, a first measure of their stability may be provided by their behavior as isolated cells in culture, and after aggregating with each other. Some of the first investigations of the behavior of isolated cells have revealed system-independent stability in isolated notochord cells (Mookerjee, Deuchar and Waddington, 1953) and heart muscle cells (Rinaldini, 1954; Cavanaugh, 1955). In those instances where the differentiation of a cell type is most readily described by its relations with other cells (for example, tubule cells) study of isolated cells is inadequate. Here we must allow cells of a particular tissue type to aggregate and reassociate. We may then observe whether they re-constitute the original tissue system. When dissociated chick mesonephros cells are allowed to aggregate they form characteristic tubular tissue in culture (Moscona and Moscona, 1952; Trinkaus and Groves, 1955). Similarly, aggregates of dissociated wing mesenchyme may form hyaline cartilage and myoblasts (Moscona and Moscona, 1952). These results certainly indicate a remarkable capacity of these cells to reestablish their tissue system after dissociation. However, inasmuch as the dissociated cells were derived from the whole mesonephros, consisting of epithelia, connective tissue, and vascular tissue, and the whole mesodermal core of the wing bud, we cannot be certain that differentiation is entirely specific on a cell-to-cell basis, kidney tubules, for example, arising only from former epithelial cells. To provide critical evidence on cell origin in these aggregates it will be necessary to use cells from a single tissue (if possible) or devise methods of marking cells.

If we are to test effectively the capacities of tissue cells to change type, we must not only release them from their tissue system but must in addition expose them to new morphogenetic stimuli. Since the possibility of such stimuli being present in the preceding experiments is remote, we must seek other means. Though greatly limited by our poor understanding of the physico-chemical basis of cell organization and the mechanism of induction, and consequently confined to rather crude methods, we may nevertheless make a beginning attack on the problem. Once cells are released from the controls of their own tissue system by dissociation, they may be subjected to new controls by combining them in culture with dissociated cells of a distinctly different tissue, in a mixed cell aggregate. In such a complex aggregate the contact or close proximity of diverse tissue cells while they are differentiating could conceivably result in significant interactions.

In their important experiments with mixed aggregates of dissociated cells from various germ layers of amphibian gastrula and neurula stages Holtfreter (1944) and Townes and Holtfreter (1955) have observed differentiation of the different tissue types to occur regionally—epidermis and endoderm at the surface, neural and mesodermal structures inside. Townes and Holtfreter (1955) interpret these results as due exclusively to a sorting out of tissue-specific cell types by directed cell movements. They base this conclusion on the observation that the pigmentation of ectoderm cells makes it possible to distinguish them from other types, and on the assumption that cells of the neurula stage are "no longer responsive" to inductive influences (Townes and Holtfreter, 1955, p. 92). In view of our limited knowledge of potency on the cell level this assumption seems unwarranted. Accordingly, in aggregates of ectoderm and mesoderm where there is a demonstrated tendency for pigmented prospective epidermis cells to move outward and for non-pigmented mesoderm cells to move inward the differentiation of epidermis from ectoderm seems established, but the specific tissue fate of the individual mesoderm cells remains an open question. The possibility that there are changes in cell type in the mesoderm cells has not been excluded.

In our own approach to this problem (Trinkaus and Groves, 1955) we have employed tissues in more advanced stages of differentiation—3½ and 5-day chick mesonephros and wing bud. Mixed aggregates of cells from these organs differentiate into areas of mesonephric tubules, cartilage, loose mesenchyme, and keratinized epithelium, in characteristic regional arrangement. The results with amphibian material suggest that this regional differentiation may be due to directed cell movements. However, certain preliminary results suggest that transformations of cell type may also be occurring, namely, a tendency in our experiments for the younger stage tissue to predominate in mixed cell aggregates from tissues at different stages of development, and a direct correlation of the number of tissues differentiating with the size of the explant. It is apparent that the possibilities of the mixed aggregate method for studying the capacities of tissue cells are great. We may not only vary the tissues combined (cf. Wolff, 1954) but vary the relative number of cells and the relative degree of differentiation as well. But before definite conclusions can be drawn on the critical matter of cell origin we must devise an adequate means of marking cells, and reduce the number of tissue types involved. With a simple technique for production of cell clones available (Puck and Marcus, 1955), the possibility of dealing with pure strains of tissue cells now emerges as a practical possibility.

The efficacy of chemical shock treatment in increasing or preserving the potencies of embryonic cells (e.g., Yamada, 1950; Gallera, 1953) suggests an added approach that may prove rewarding. If dissociated tissue cells were subjected to such a treatment (cf. Weiss and James, 1955), it is conceivable that their type-specific organization would be so altered as to create greater responsiveness to new inductive influences. If these cells were then mingled with cells of another tissue type in a mixed aggregate, directed changes in cell type might occur. Although ignorance forces us to thread our way in a largely empirical manner, there is some reason to suspect that use of agents which interfere with protein metabolism would be fruitful. In view of the probability that the effect of urea on the competence of ventral ectoderm (Gallera, 1953; Gallera and Baud, 1954) is due to its well established activity as a mild depolymerizing agent for proteins (Neurath, et al., 1944), use of other such agents would be in order. Moreover, because of the close relation between protein and nucleic acid metabolism (Gale and Folkes, 1954), factors that interfere with the latter may also affect proteins. For example, Lallier (1954) has found that lithium chloride causes both a loss of ribonucleic acid and an inhibition of glycolytic activity in amphibian gastrulae. Presumably these metabolic effects of lithium are related to its effect on development. Probings such as these may open a wide and instructive area of investigation.

SUMMARY

The evidence for the theory of irreversible differentiation of tissue cells is examined and found to be inconclusive. On the basis of this analysis:

a) another, more tentative definition of differentiation on the cellular level is proposed, and b) suggestions are made for future research on the stability of tissue cells.

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CARYONIDAL INHERITANCE AND NUCLEAR DIFFERENTIATION*

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Caryonidal inheritance of mating types in *Paramecium aurelia* was first reported by Sonneborn in 1937 and its relationship to problems of development was noted in 1947(b), but the significance of these observations and speculations has not been generally appreciated. Caryonidal inheritance implies that somatic nuclei may differentiate, that is, that nuclei with the same genetic elements are modified to control different phenotypes and that these nuclear modifications are "hereditary." This implication bears significantly on the problem of cellular differentiation, but has been largely neglected—chiefly perhaps because of a lack of corroborative evidence from other forms. The classical tests for nuclear differentiation during early stages of development were uniformly negative. Recently, however, strong evidence for nuclear differentiation at somewhat later stages has been provided through the work of King and Briggs (1955). It now appears that studies on caryonidal inheritance in the Ciliates may provide model systems of general interest.

The essential feature of caryonidal inheritance is the correlation between certain cytogenetic phenomena and the distribution of traits among the progeny of single conjugating pairs. To facilitate discussion these cytogenetic events and their associated terminology are presented diagramatically in an abbreviated form in figure 1. The details of these processes vary from species to species and those in figure 1 are based specifically on *Tetrahymena pyriformis* (Elliott and Hayes, 1953; Nanney, 1953; Ray, 1954). Conjugating pairs are formed when cells of diverse mating type are brought together under appropriate conditions. Following a series of prezygotic nuclear events (including meiosis, formation and transfer of gamete nuclei), syncarya of identical constitutions are established in the two members of a pair. The postzygotic nuclear events (involving nuclear division, differentiation and disintegration) result in the establishment in each exconjugant of two macronuclei and a single micronucleus. These two macronuclei are separated at the first cell division following conjugation and each of the cells produced at this division gives rise to a culture designated as a *caryonide*. The two caryonides derived from a single exconjugant are called *sister caryonides;* two caryonides from different members of

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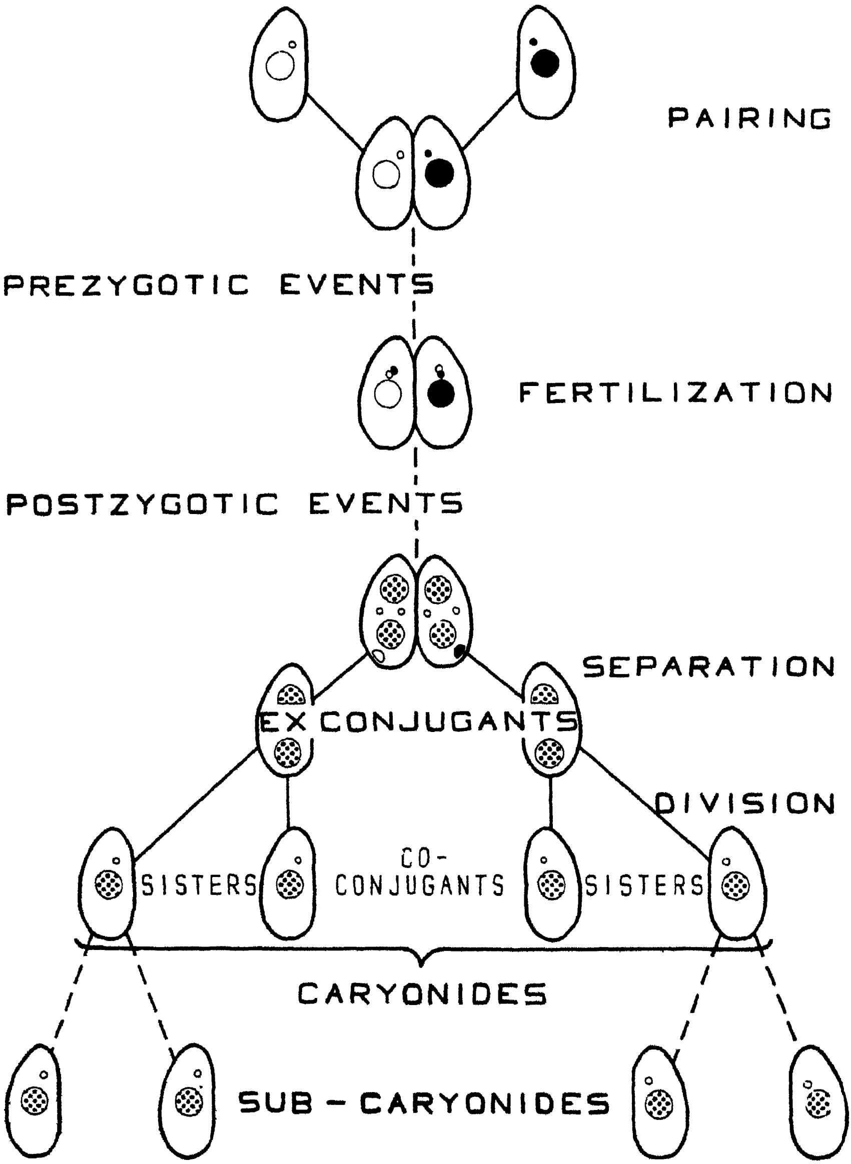

FIGURE 1. Abbreviated representation of events occurring during and after conjugation in *Tetrahymena pyriformis*. (See Text)

the same pair are called *co-conjugant caryonides*. Subcultures derived from a single caryonide at any time after its origin are designated as *sub-caryonides*. The nuclei in the cells derived from a single conjugating pair contain the same genes.

In spite of the genetic identity of the cells derived from a single pair, persistent mating type diversities appear among them. Caryonidal inheritance is indicated by a particular pattern in the origin of these mating type diversities, that is, by a particular distribution of mating types among the cultures derived from single conjugating pairs. The details of this pattern vary from species to species, but one feature remains constant: sub-cultures of a single caryonide usually manifest the same mating types, while sister caryonides or co-conjugant caryonides often manifest different types. A high correlation is thus observed between the time of separation of new macronuclei (at the first cell division following conjugation) and the time when diverse cell lineages originate. The simplest interpretation of this correlation attributes mating type control to the macronuclei and implies that nuclei with the same genetic constitutions may differentiate so as to control different traits. Sonneborn (1947a, 1954) has presented several other lines of evidence which support this conclusion.

This paper documents certain features of caryonidal inheritance in variety 1 of *Tetrahymena pyriformis* and examines the significance of this system in assessing the role of the nucleus in cellular differentiation.

MATERIALS AND METHODS

The strains used in this study were derived from two cells isolated by Elliott and Gruchy (1952) from a pond near Woods Hole, Massachusetts, and designated as WH-6 (mating type I) and WH-14 (mating type II). Crosses between these strains and among their progeny yielded a total of seven mating types which mate in all combinations of different types (Nanney and Caughey, 1953). The reactions among these seven types produce the breeding system shown in table 1.

TABLE 1

THE BREEDING SYSTEM IN VARIETY 1 OF *TETRAHYMENA PYRIFORMIS*. THE PLUS SIGNS INDICATE THE OCCURRENCE OF CONJUGATION; THE MINUS SIGNS INDICATE A FAILURE TO CONJUGATE.

Mating Types	I	II	III	IV	V	VI	VII
I	–	+	+	+	+	+	+
II		–	+	+	+	+	+
III			–	+	+	+	+
IV				–	+	+	+
V					–	+	+
VI						–	+
VII							–

Upon inbreeding, the original strains gave rise to two diverse lines of descent, distinguished by the assortment of mating types they produce when crossed among themselves. These derived strains are designated as "Family A" and "Family B." Types I, II, III, V and VI appear in characteristic frequencies after conjugation within Family A; types II, III, IV, V, VI and VII occur regularly in Family B. A genetic analysis (Nanney, Caughey and Tefankjian, 1955) demonstrates that the distinctions between these families are due to differences in a single chromosome. Family A is homozygous for an allele, $mt^{(IV, VII)-}$ and Family B is homozygous for an allele, $mt^{(I)-}$. Not only the mating type *potentialities*, but also the mating type *frequencies*, are strongly influenced by these genetic factors, as may be seen in the data tabulated in table 2. Although these effects appear to be related to a single chromosomal locus, sufficient data are not available to exclude several closely linked loci. The complexity of the differences in fact suggests a more complicated genetic basis.

Most of the methods used in this work have been presented elsewhere (Nanney and Caughey, 1955; Nanney, Caughey and Tefankjian, 1955) and will not be discussed in detail. It is necessary, however, to mention the methods used to establish that conjugation has in fact occurred. Some pairs separate without undergoing a complete nuclear reorganization, undergo no change in mating type, and do not enter a period of sexual immaturity. Screening for such pairs has been accomplished by several procedures, some of which are not satisfactory for an analysis of mating type distribution. Particularly, some crosses have been screened simply on the basis of the mating types of the derived cultures. If the two "caryonides" from one pair member were of the same mating type as one of the parents and the two caryonides from the other pair member corresponded in mating type to the other parent, the pair was considered non-conjugant. It should be apparent that this technique eliminates non-conjugants (except in the unlikely event that one or both parents were unstable in their mating type [Nanney and Caughey, 1955]), but it also eliminates some of the conjugants and distorts the mating type distribution. Previously published data were taken from crosses which were not screened at all and are, hence, subject to a different kind of bias.

TABLE 2

GENOTYPIC INFLUENCES ON MATING TYPE POTENTIALITIES AND FREQUENCIES IN VARIETY 1 OF *TETRAHYMENA PYRIFORMIS*.

Genotype	Mating Type Frequencies							Total
	I	II	III	IV	V	VI	VII	
$mt^{(I)-}/mt^{(I)-}$ (Family B)	.000	.174	.083	.534	.038	.073	.123	397
$mt^{(IV,\ VII)-}/mt^{(I)-}$ (Hybrid)	.115	.135	.145	.236	.070	.178	.120	399
$mt^{(IV,\ VII)-}/mt^{(IV,\ VII)-}$ (Family A)	.219	.160	.155	.000	.074	.391	.000	2350

A more reliable procedure has been used exclusively in the experiments to be reported and is based on the observation that true conjugants are immature following conjugation. The duration of the immature state varies considerably depending upon the environmental conditions and the particular parents used in the cross, but in the crosses reported here no evidence could be found for true conjugants maturing before the 20th fission after conjugation. The maturity tests and associated manipulations are conducted as follows. Conjugating pairs are isolated into depression slides. After 10–12 hours, when most of the pairs have separated and when none of the exconjugants has divided, the two members of a pair are isolated into different depression slides. During the next 12 hours these cells are periodically examined and the daughter cells are separated after the first fission. This procedure yields four depression cultures (caryonides) from each conjugating pair. Under our conditions (26 C. in Cerophyl inoculated with *Aerobacter aerogenes*) the cultures undergo 8–10 fissions in 24 hours and exhaust the medium on the second day, after having undergone a total of 12–13 fissions. These cultures are perpetuated by making single cell transfers to new depressions, usually on alternate days, until they have completed growth in seven depression cultures, and are then tubed. Those depression cultures remaining after new isolations have been made are designated as "left-over" cultures. The first left-over culture is tested for maturity.

Maturity tests are made by adding an approximately equal amount of a well-fed culture of a mating type unlike either of the parents and preferably of a type which the cross could not produce. On the following day the mixture has starved and the cultures have mated if they are mature. Those clones classified as non-conjugant on the basis of maturity tests are immediately tubed (from the second isolation culture) and tested for mating type, thus providing an additional test for non-conjugation.

One other source of possible bias must also be mentioned. Exconjugant cells are much smaller than vegetative cells and may remain quiescent for long periods of time. These cells often settle among the bacteria in the center of a depression slide and are difficult to find. Moreover, the time of division of the two exconjugants of a pair may differ by as much as several hours. It is conceivable that occasionally the products of a first division are confused with the exconjugants and that the subsequently separated "caryonides" are in fact sub-caryonides. This possibility of error is increased by the occasional occurrence of unilateral death in most crosses. The death of one of the fission products is also not a rare event and introduces a similar source of error. Although the *degree* to which these considerations may bias the results is not known, the *direction* of the bias is clear. Since sub-caryonides tend strongly to be alike in mating type, the mistaken identification of sub-caryonides as caryonides would produce an apparent correlation in the mating types of sister caryonides, even if none existed. On the other hand, this bias might eliminate a minor negative correlation among sister caryonides.

RESULTS

1. *Mating type distribution.* The first question to be considered concerns the distribution of mating types among the cultures derived from a single pair. Ideally an analysis of mating type distribution would be accomplished by classifying each pair according to the mating types observed in the four caryonides, but the necessary number of pairs is not yet available. The

large number of mating types in this system gives a very much larger number of classes of pairs (120 distinguishable classes within Family A alone), some of which are reasonably common and some of which are very rare. Moreover, the death or loss of some of the caryonides in a pair renders that pair useless in such an analysis; many such "incomplete" pairs are available and should be used if possible. The data now available can be used to good advantage simply by making comparisons between paired cultures: sister sub-caryonides, sister caryonides and co-conjugant caryonides.

The most essential element in a system of caryonidal inheritance is the relative homogeneity *within* caryonides as compared with the diversity *between* caryonides. The distribution of mating types within sub-caryonides derived prior to the 10th postzygotic fission is shown in table 3 for Family A and table 4 for Family B. Although differences occur within single caryonides and although many combinations of types occur, sister sub-caryonides are strongly predisposed to be alike. The observed differences are probably due to the occurrence of unstable caryonides from which different pure types have been derived (Nanney and Caughey, 1955). No striking tendency for any combination of types, other than like types, is apparent, but the data are not sufficiently numerous to exclude such a tendency and a thorough treatment of this problem will be deferred for later publication.

TABLE 3

MATING TYPE COMBINATIONS IN PAIRED CULTURES FROM SINGLE CONJUGATING PAIRS IN FAMILY A

Combinations	Sub-caryonides	Sister Caryonides		Co-conjugant Caryonides	
	Observed	Observed	Expected	Observed	Expected
I-I	20	20	20.9	39	39.5
I-II	0	31	33.4	69	64.0
I-III	4	32	34.1	42	51.3
I-V	3	13	12.7	25	24.5
I-VI	6	78	72.3	141	135.0
II-II	33	11	13.4	20	25.5
II-III	2	31	27.5	50	41.0
II-V	0	13	10.0	16	19.8
II-VI	1	59	57.7	110	109.0
III-III	30	22	14.1	19	16.6
III-V	2	7	13.2	17	15.8
III-VI	4	46	59.5	80	87.0
V-V	9	3	1.9	9	3.7
V-VI	1	20	22.0	33	41.8
VI-VI	34	68	63.2	120	115.5
Totals	149	454		790	
Chi Square		11.4		6.5	
D. F.		9		9	
P		.2-.3		.7-.8	

A comparison of the mating types of sister caryonides reveals no such tendency to identity (tables 3 and 4). The frequencies of the combinations correspond well with the frequencies calculated on the basis of chance. These expected values were calculated as follows. The frequency of each type was derived from the entries in the column, thus yielding a frequency, p, for mating type I, q for mating type II, r for mating type III, etc. The probabilities for various combinations of types were derived from these frequencies; thus the probability for two mating type I cultures is p^2, for two cultures of mating type II is q^2, etc.; the probability for one type I and one type II is $2pq$, for one type I and one type III is $2pr$, etc. Although the

TABLE 4

MATING TYPE COMBINATIONS IN PAIRED CULTURES FROM SINGLE CONJUGATING PAIRS IN FAMILY B

Combinations	Sub-caryonides	Sister Caryonides		Co-conjugant Caryonides	
	Observed	Observed	Expected	Observed	Expected
II–II	34	8	2.5	6	5.5
II–III	1	2	3.6	5	4.6
II–IV	2	27	26.2	37	34.2
II–V	0	0	2.0	1	2.6
II–VI	1	2	3.7	4	4.8
II–VII	0	7	6.0	7	7.9
III–III	9	2	1.1	4	1.4
III–IV	2	11	13.5	15	17.6
III–V	0	3	1.0	0	1.4
III–VI	1	1	1.9	2	2.5
III–VII	5	6	3.1	4	4.0
IV–IV	87	43	42.2	58	55.2
IV–V	0	5	6.5	7	8.5
IV–VI	6	11	11.9	12	15.6
IV–VII	7	18	19.3	23	25.0
V–V	5	0	0.6	0	0.3
V–VI	0	2	1.0	4	1.2
V–VII	0	1	1.5	4	1.9
VI–VI	4	2	1.1	1	1.1
VI–VII	3	3	2.7	6	3.5
VII–VII	14	1	1.8	2	2.8
Totals	181	155		202	
Chi Square		6.6		5.3	
D. F.		5		5	
P.		.2–.3		.3–.5	

pairs used in this analysis were derived from crosses of clones from many different sources, the mating type combinations within pairs are essentially random.

An analysis of mating type combinations in co-conjugant caryonides produces similar results (tables 3 and 4). When all four caryonides are available, four different comparisons of co-conjugant caryonides are possible—each of the caryonides from each pair member with each of the caryonides from the other member. The first three comparisons, however, uniquely determine the fourth, and the first two comparisons partially determine the third. For this reason only two comparisons were made in tabulating the data: one preselected caryonide from the first pair member with one preselected caryonide from the second, and the other caryonide from the first with the other caryonide from the second. Again close agreement is found between the frequencies of mating types observed and the frequencies expected. We are, therefore, led to the conclusion that the mating types developed by macronuclei within a single pair (or within a single exconjugant) are determined more or less independently.

TABLE 5

THE RELATIONSHIP BETWEEN THE MATING TYPE OF THE PARENT AND THE MATING TYPE FREQUENCIES IN THE PROGENY WITHIN FAMILY A

Parents	Crosses		Mating Type Frequencies					
			I	II	III	V	VI	Total
I, II	6	No.	46	39	33	16	96	230
		%	.200	.169	.143	.070	.417	
I, III	2	No.	43	32	22	20	90	207
		%	.208	.155	.106	.079	.445	
I, V	2	No.	37	13	16	9	78	153
		%	.242	.085	.104	.059	.510	
I, VI	11	No.	106	70	90	37	165	468
		%	.227	.150	.192	.079	.355	
II, III	2	No.	37	22	18	3	83	163
		%	.227	.135	.110	.018	.510	
II, V	4	No.	38	24	24	19	57	162
		%	.234	.148	.148	.117	.358	
II, VI	7	No.	67	65	72	24	98	326
		%	.206	.199	.221	.074	.301	
III, V	3	No.	80	56	32	13	125	306
		%	.263	.183	.109	.043	.409	
III, VI	4	No.	28	22	24	16	54	144
		%	.194	.153	.167	.111	.375	
V, VI	6	No.	35	33	34	16	73	191
		%	.183	.173	.178	.084	.383	
Total		No.	517	376	365	173	919	2350
		%	.219	.160	.155	.074	.391	

2. *Parent-progeny relationships.* The next question to be considered involves the relationship between the mating type of the parent and that of the progeny. In *P. aurelia* this problem can be explored by relatively simple procedures (Sonneborn, 1947a, 1950). Uniparental nuclear reorganization (autogamy) occurs periodically in all the stocks and presents no problem of interpretation regarding the mating type of the parent. Moreover, cytoplasmic "markers" are available which permit the identification of the cytoplasmic parent even when conjugation occurs in mixed cultures. Autogamy has not been found in *T. pyriformis*, however (though it occurs in a

related species, *T. rostrata* [Corliss, 1952]), and cytoplasmic markers are not yet available. Hence, less direct procedures must be used.

The method adopted was to make crosses among a series of cultures which differ in mating type and to examine the frequencies of the mating types among the progeny. If the parental mating type has an influence on the mating type of the progeny, significant and systematic differences in mating type frequencies would be expected in different crosses. The results of such crosses within Family A are shown in table 5. First of all, it is apparent that no *qualitative* distinctions are introduced as a result of variations in parental types; the same types appear among the progeny and in roughly the same proportions. Significant deviations do appear, however, in the relative frequencies of the types (P value of less than .001 for a chi square test for homogeneity). Certain patterns in the deviations can also be detected. Thus fluctuations in mating type III are approximately compensated by fluctuations in mating type VI; when types III and VI are combined into a single class, the homogeneity of all crosses is increased (P = .05-.10). These deviations, moreover, appear to be related to the appearance of these same types among the parents; when type III is in the parents, type III is usually depressed in the progeny; when type VI is in the parents, type VI is usually depressed among the progeny. These observations suggest a slight negative parent-progeny correlation, but the data are not convincing on this point.

The heterogeneity within table 5 may have been brought about by factors other than parental mating types. Although the parents were all homozygous for the $mt^{(IV,\ VII)-}$ allele, they may have become genetically diverse at other loci as a result of inbreeding. The crosses were performed with clones from several different inbred sub-lines and certain types of crosses occurred more often in some sub-lines than in others. Secondly, slightly different growth conditions were employed in some of these experiments and the treatments were not properly randomized. The progeny from some crosses were grown at maximal rate and yielded many selfers; other progeny were starved intermittently and produced largely stable cultures. The failure to control these variables was based on initial small scale experiments which showed no significant effect of these factors on mating type determination. In view of the slight deviations noted in the much larger samples, the initial experiments cannot be considered decisive and further studies will have to be undertaken to determine the cause of the variations. Modifying genes and environmental circumstances, as well as parental phenotypes, must be considered.

DISCUSSION

1. *Patterns of Caryonidal inheritance.* Caryonidal inheritance of mating types has been demonstrated in two genera of the Ciliated Protozoa—Paramecium and Tetrahymena. Although agreeing in many essential features, various patterns of caryonidal distribution can be distinguished in these forms. The simplest pattern occurs in the Group A varieties of *P.*

aurelia (Sonneborn, 1937, 1947a; Kimball, 1937). Within a single variety only three classes of caryonides appear, those pure for one of the possible mating types, those pure for the other type, and unstable cultures which contain both mating types. Most of the study of this system has been concentrated on those strains and varieties in which mating type instability is rare or absent, but the available information on the other strains suggests no major modifications. The distinguishing features of this system are 1) a lack of any correlation between the mating types of parents and progeny, 2) a lack of any correlation between the mating types of sister caryonides or of co-conjugant caryonides, 3) a marked effect of temperature during the period of macronuclear development on the mating type frequencies, and 4) genetic control of mating type potentialities (Sonneborn, 1947a) and mating type frequencies (Butzel, 1955).

The observations on the Group B varieties of *P. aurelia* indicate the operation of a very different sort of system (Sonneborn, 1947a). The chief characteristics of this system are 1) a strong positive correlation between the mating type of the parent and that of its sexual progeny; 2) a strong positive correlation between the mating types of sister caryonides and a strong negative correlation between the mating types of co-conjugant caryonides when no cytoplasmic fusion occurs; 3) a strong positive correlation between co-conjugant caryonides when extensive cytoplasmic fusion occurs; 4) a slight temperature effect on mating type frequencies under normal conditions, but a marked effect when cytoplasmic fusion occurs (Nanney, 1954); and 5) no known effect of nuclear genes on mating type potentialities and only suggestive evidence for genetic effects on mating type frequencies. In spite of the differences between the Group A and Group B patterns, Sonneborn (1954) has clearly established that the macronuclei as certainly control the mating types in the B as in the A Group. The chief distinction between the groups is in the existence of a cycle of nucleo-cytoplasmic interaction in the B system; the macronucleus not only controls the mating type, but it is also responsible for establishing in the cytoplasm a mechanism for inducing new macronuclei to develop in the same manner as the old one (Nanney, 1954).

Studies on the system of mating type determination in *P. bursaria* are beset with many difficulties, but sufficient information is available to suggest a pattern of caryonidal inheritance related to that in the Group B varieties of *P. aurelia*, but involving multiple mating types (Jennings, 1942). The significant observations are as follows. 1) The progeny of a single pair usually have the same mating type, but when they differ, caryonidal distribution may occasionally be observed. 2) A cross of any two mating types *may* produce—in different pairs—all the mating types in that variety, but the parental types are usually in excess in the progeny. 3) Crosses of the same types derived from different sources may produce different spectra of mating types or the same types in different frequencies. 4) Cytoplasmic exchange probably occurs regularly during conjugation (Harrison and Fowler, 1945). The rarely observed differences between sister caryonides suggest, but do not unequivocally establish, caryonidal determination. The parent-progeny correlation suggests a cytoplasmic mechanism similar to that found in the Group B varieties, but modified by the regular occurrence of cytoplasmic exchange and the confluence of antagonistic cytoplasmic systems at the time of conjugation. The variations in crosses of strains from different sources suggest genetic determination of potentialities and frequencies. This interpretation of mating type determination in *P. bursaria* differs substantially from that proposed by Jennings, but is in essential agreement with a later interpretation by Sonneborn (1947a). One observation concerning the *P. bursaria* system (Jennings, 1941) is not precisely matched in the other systems and concerns the regular establishment of semi-stable conditions in which two types replace each other sporadically in the course of growth.

Finally, the caryonidal system found in Tetrahymena represents yet a different type of pattern, operating—like the *P. bursaria* system—with multiple mating types, but in a manner most similar to that in the Group A varieties of *P. aurelia*. No significant correlations have been established between sister or co-conjugant caryonides. As in the Group A varieties, a temperature effect (Nanney and Caughey, 1953) has been found on mating type frequencies and genetic influences on mating type potentialities and frequencies have been noted (Nanney, Caughey and Tefankjian, 1955).

2. *The nature and significance of nuclear differentiation.* That certain types of nuclear differentiation occur has long been established. Chromosome diminution, polyploidy, aneuploidy or polyteny are known to occur in a variety of tissues in many organisms. These nuclear alterations do not, however, occur in enough organisms and do not offer sufficient variety to play a predominant role in developmental differentiation. Moreover, *initial* polyploidy and aneuploidy do not change the main course of development. The striking morphological changes occurring during nuclear development in the Ciliates (Nanney, 1953; Sonneborn, 1954) probably have no direct parallel in the development of higher forms. Recently, characteristic enzymatic patterns have been established for nuclei in different tissues (See Allfrey *et al.*, 1955), and characteristic chromosomal changes associated with differentiation have been reported (Pavan and Breuer, 1952), but such studies fail to distinguish between nuclear differentiation as a consequence and nuclear differentiation as a causal factor in developmental alterations. Relatively few experimental procedures in fact permit examination of the heritability of nuclear modifications as opposed to the heritability of the cellular conditions as a whole. In spite of this, most attempts to develop models of differentiation have focussed attention on the cytoplasm.

Two kinds of evidence have chiefly been responsible for the belief that cellular differentiation is primarily a cytoplasmic function. The first of these was that leading to the establishment of the chromosome theory of inheritance. The chromosomes were shown to be the vehicles of inheritance and the precise and equal distribution of the chromosomes at most cell divisions was demonstrated. Hence, the genetic (hereditary) characteristics of the nuclei must as a rule remain constant and hereditary differences characterizing cell lineages during development must have a cytoplasmic basis. It should be pointed out, however, that the observations contradict merely the concept of a *gross* partition of the chromosomal elements among differentiated tissues; they do not eliminate subtle metabolic or structural variations in the nuclei, or patterns of activation and inactivation of genetic loci without material loss. Cytoplasmic systems of heredity were postulated as the alternative to gross modification or segregation of chromosomal material, and without sufficient regard for other types of nuclear alteration.

The second kind of evidence was that obtained from studies in experimental embryology, particularly those of the kind conducted by Spemann (1938). Constriction of a fertilized amphibian egg into two portions—one with and one without a nucleus—allowed the multiplication of the nucleus for several cell divisions in one half of the egg. The subsequent escape of one of the daughter nuclei to the other half provided an excellent test for the hypothesis of heritable nuclear differentiation. If the nuclei had irreversibly differentiated during the early cleavage stages, development should be aberrant in the half of the egg with only one of the many daughter nuclei. This was not observed. Hence, it was concluded that irreversible (heritable) nuclear differentiation did not occur, at least during the early cleavage stages. Technical limitations prevented similar studies at later stages. Recently, however, Briggs and King (1953) have developed techniques for extending such studies. Nuclei (with some associated cytoplasm) have been removed from the cells of blastulae, gastrulae and later stages and injected into enucleated eggs. Their results with nuclei which have undergone relatively few divisions confirm Spemann's conclusions, but with successively later stages, fewer and fewer nuclei were found capable of sustaining normal development when transferred. Finally they have established the heritability of nuclear alterations through serial transfers of differentiated nuclei (Briggs and King, personal communication). If taken at face value these results require a rejection of the persistent totipotentiality of nuclei and a reassessment of the role of the nucleus in development. The model systems based primarily on cytoplasmic modifications may have less significance in developmental differentiation than usually supposed.

The first problem raised is the nature of the nuclear modification. Gross changes in nuclear constitution may be eliminated as not occurring with sufficient regularity or frequency. Secondly, traditionally conceived "mutations" must be rejected. If nuclear alterations are to account for any major part of cellular differentiation, they must differ from mutations—not in their heritability in general or in any one specific characteristic, but in a constellation of characteristics. 1) They must occur in much higher frequency. 2) They must not be distributed randomly in time. 3) They must occur as specific responses to specific stimuli. 4) Their variety must be sharply circumscribed by the original genetic constitution of the cell. These characteristics do not imply that the nuclear alterations are extra-chromosomal or that they can in every case be easily distinguished from gene mutations, but only that—as a class—developmental modifications differ significantly from mutations in the usual sense of the word.

Several model systems of nuclear differentiation may be developed on the basis of the work in the Ciliates. These formulations rely heavily on efforts to develop coherent cytoplasmic systems and indeed show various degrees of integration with those systems. The first model to be examined is one which might be pertinent to the Group A caryonidal system in *P. aurelia*, or to that in variety 1 of *T. pyriformis* (figure 2A). The various genes controlling alternative potentialities would initiate a series of reactions resulting in gene products. (The actual number of genes involved is not relevant.) These gene products might then interact, within the nucleus, in a manner similar to that proposed by Delbrück (1949) for a cytoplasmic "steady state." The result of this antagonistic interaction would be the establishment of one series of reactions and the inhibition of the others. Some terminal or sub-terminal reaction product from the prevailing reaction series could be transmitted through the cytoplasm and translated into a phenotypic specificity at the surface of the cell. Since the antagonistic reactions are conceived as restricted to the nucleus, they should take independent courses in different nuclei developing in the same cell.

One modification transforms this system from that characteristic of the Group A varieties to that for the Group B varieties. (figure 2B.) Instead of restricting the antagonistic reactions to the nucleus, these could "overflow" into the cytoplasm and influence newly developing nuclei to imitate the same series of reactions. It should be pointed out that the efficiency of this system of inter-nuclear "communication" determines whether such systems can be identified as "caryonidal." If the transfer through the cytoplasm is perfectly efficient, the role of the nucleus is obscured and "inheritance" appears to be strictly cytoplasmic. The Group B system was in fact long considered such a system and only recently has the role of the nucleus been clarified. The serotype system in *P. aurelia* (Beale, 1954) shares many of the properties of the Group B mating type system. Although no evidence is thus far available to indicate caryonidal inheritance, this system may be conceptualized as a slightly more efficient model of the Group B pattern. One other feature of the serotype system also distinguishes it from the mating type system, that is, the relative ease of reversing a differentiation. This is not, however, a qualitative difference, since mating type instability *can* also exist for long periods of time.

In any case, no major problem arises in developing models of varying degrees of stability and the degree of stability of a system might well be influenced by the requirements of the cell. Thus a unicellular organism is

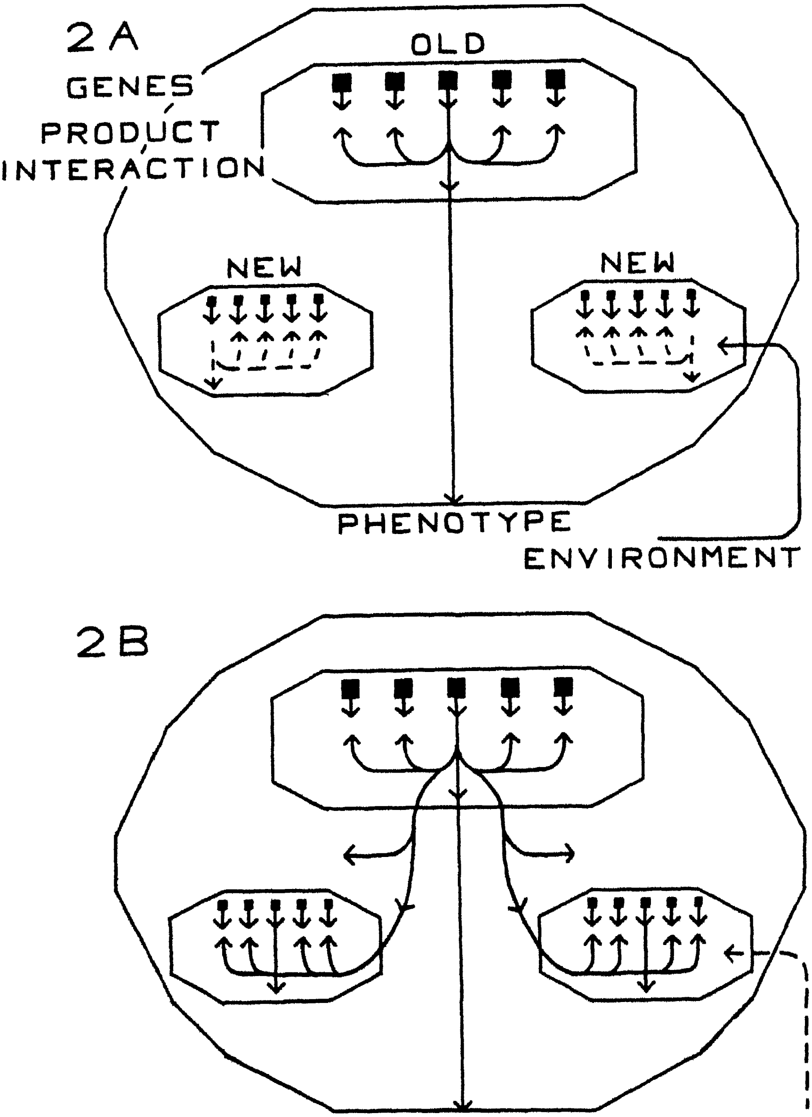

FIGURE 2. Two models of nuclear differentiation based on studies in the Ciliated Protozoa. (See Text)

to a large extent self sufficient, performing all the functions required for its existence; it cannot afford to relinquish as many potentialities as a metazoan cell, which participates in a system of symbiotic cellular interactions. Cellular differentiation in a unicellular organism in most cases would afford adaptation to particular vicissitudes in the environment, but the retention of plasticity—an ability to return to a former condition when the environment changed—would be beneficial. For this reason one might expect differentiation in unicellular organisms to be more freely reversible than that in higher organisms. In the mating type system no hint is yet available concerning the selective advantage of a particular mating type for a cell considered as an independent entity, but all the advantages derive from the interactions of the cells in the population as a whole. In this particular case, therefore, selective advantage is contingent upon cellular interactions at the population level and not upon adaptability at the cellular level. Hence, the relative irreversibility of mating type differentiation may set it apart from most systems of differentiation in unicellular organisms and consequently may make it closer to systems of differentiation in metazoans.

These models do not involve alterations in the genes, the chromosomes or in their primary activities, but rather they assume steady state systems either localized within the nucleus or spilling over from the nucleus into the cytoplasm. It is of course conceivable that such a system could be entirely localized in the cytoplasm, in which case no evidence for nuclear differentiation would be available. On the other hand, alterations could occur at the level of the chromosomes themselves. Lederberg and Lederberg (1955) report evidence for persistant antigenic variations in Salmonella associated with reversible modifications at transducible genetic loci, and McClintock (1951) has studied what may be similar modifications in maize. It should be apparent, however, that *integrated* development, involving many different genetic loci, is scarcely conceivable without some form of intra-nuclear communication and coordination; steady state systems offer attractive possibilities in this regard. Fortunately, at this beginning of a reassessment of the role of the nucleus no immediate decision among the various models is required and no reason exists for believing that all nuclear differentiation involves the same mechanism.

Finally a word of caution is required regarding the organisms upon which these models are based. It is possible that the peculiarities of Ciliate structure provide patterns of cellular heredity far from typical. Most concern is developed in regard to the macronucleus, which differs in many respects from conventional nuclei. 1) It contains many sets of chromosomes, but their organization within the nucleus is an unsolved problem. The macronucleus may be composed of balanced sub-nuclei (Sonneborn, 1947a) or it may be a highly disorganized polyploid nucleus (Kimball, 1953). It must have mechanisms for the orderly distribution of genetic material at cell division but these are not understood. In any case, the problem of intra-nuclear coordination is more acute here than in simple nuclei and may have resulted in novel solutions. 2) The nuclear membrane in these forms does not break down at cell division as it does in most forms. This failure of breakdown may be conducive to the establishment of "nucleoplasmic" steady states which would be dissipated ordinarily at cell division. One might expect conventional cells to develop systems of cellular heredity more closely associated with the chromosomes—so as to persist through cell division—or to extend such systems more consistently through the cytoplasm. These problems cannot be solved, however, until more is known about cellular heredity, not only in the Ciliates but in other organisms.

SUMMARY

The following features of caryonidal inheritance in variety 1 of *Tetrahymena pyriformis* are documented. 1. Cells with the same genetic constitutions may develop different phenotypes, which are inherited during vegetative growth. 2. Mating type diversities which arise at conjugation are distributed approximately at random among the products of the first fission after conjugation. 3. Within a genotype no positive correlation can be detected between the mating type of the parent and the mating type of the progeny.

Following Sonneborn, these and previously published observations, both on Tetrahymena and Paramecium, are interpreted as indicating nuclear differentiation as the basis for mating type determination in these forms. Suggestions regarding the mechanisms of nuclear differentiation and the general significance of such mechanisms are set forth.

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THE SENSITIVITY OF THE GOLDFISH (*CARASSIUS AURATUS L.*) TO POINT HEAT STIMULATION

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INTRODUCTION

It is known that fish can perceive temperature differences of .03 to .05 °C (Bull, 1937) and that they have thermoreceptors in the skin (Dijkgraaf, 1940). The state of our knowledge of temperature perception in fish has recently been summarized by Sullivan (1954). This review showed again that there are a number of questions which remain to be answered about the fish's perception of this important environmental variable. For instance: what is the level of precision of the individual receptor? Are fish more heat-sensitive on certain parts of their bodies than on others?

No specialization of nerve endings was found in the skin of those teleosts of which histological studies were made (Krause, 1928). There is, however, profuse branching of fine fibers with a great number of free endings per unit area.

In order to elucidate the mode of action of thermosensory surface receptors fish were tested for the distribution and relative density of these nervous elements.

These tests suggest that goldfish, typical teleosts, rely for their fine temperature (warmth) discrimination on areal summation which operates in several instances where the functioning of vertebrate warm and cold receptors has been studied in detail (Sand, 1938; Bullock, 1953; Hardy and Oppel, 1937; and Dodt and Zotterman, 1952). Dijkgraaf (loc. cit.) interpretated some of his observations on *Misgurnus fossilis* and *Ameirus nebulosus* in a similar manner.

MATERIALS AND METHODS

The experiments involved a conditioned reflex training technique with which it could be ascertained whether or not the fish reacted to a point heat stimulus of a certain measurable intensity.

1. *Apparatus.* In a 30 gallon aquarium of 36 × 14 × 14 inches, an inner and outer chamber was built of opaque plastic. The outside of the tank was made opaque with window shade material.

A copper screen was placed on the bottom of the tank to form one electrode of an electric shocking system. Wooden frames with copper wire stretched over them functioned as the other electrode. They were fitted in the aquarium in such a manner that the experimenter could administer a mild

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[1] Grateful acknowledgement is made to the Horace H. Rackham School of Graduate Studies of the University of Michigan which made this research possible, and to Richard G. Bjorklund for most conscientious assistance.

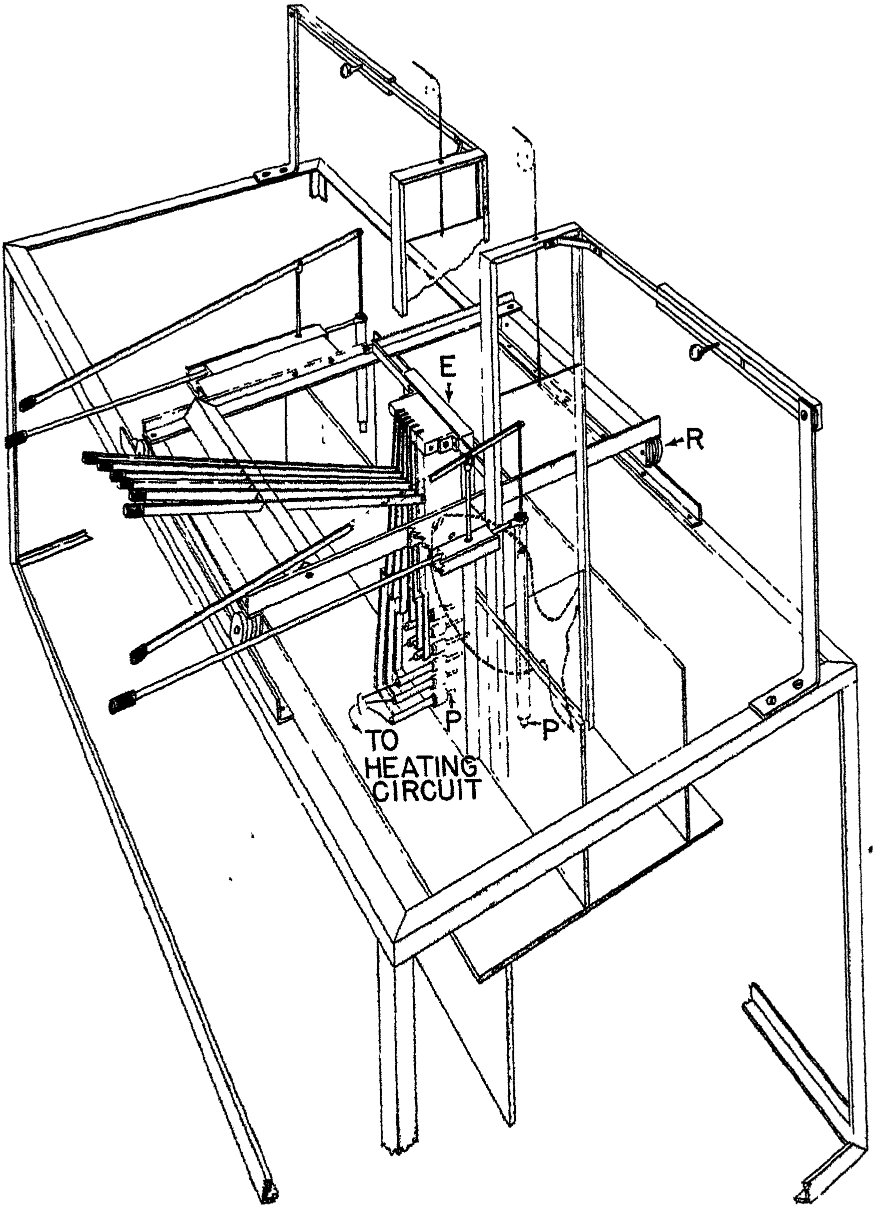

FIGURE 1. Experimental tank for spot heat stimulation of goldfish.
E. Experimental or test chamber.
P. Probes of nichrome wire.
R. Rail and rollers to shift probes along side of fish.

shock to the fish in any portion of the tank except the experimental chamber proper, which was situated in the middle of the long side of the tank distal to the observer (E, figure 1). There the heat stimulating devices made the placement of a shocking electrode difficult. The current used was 110 volts A.C., stepped down with a Variac transformer to about one to two volts depending upon the size of the fish. If a shock was to be administered in the experimental chamber itself, the current was increased slightly and the key activating the frame behind the fish was depressed.

A system of movable nichrome wire heating elements was arranged in the experimental chamber in such a way that a fish could be touched with the points of these heaters anywhere on its body (P, figure 1).

The heating elements were connected to a voltmeter and an ammeter on a selector switch circuit so that the heater wattage could be adjusted to any desired temperature with a potentiometer. Heat output curves at several wattages, from .5° to 40°C, were determined in well stirred water for each of the points of the heating probes by making contact with thermocouples of No. 30 B. and S. gauge copper constantan wire and a Leeds and Northrup Speedomax recording potentiometer. The entire arrangement of probes, and the levers to activate them were set on rails so that they could be moved along the side of the fish (R, figure 1). The action of the heating probes, each of about 2 sq. mm. surface, was individually controlled through a system of cams and levers from the outside of the aquarium.

A mirror covering the entire aquarium was placed above it at a 45 degree angle and a second smaller mirror was suspended so that the experimenter had a detailed view of the test chamber and could spot the position at which any one heating element touched the fish. The first step of the training procedure required bright light. A 100 watt light was hung over the aquarium and shaded to illuminate the tank only and leave the observer in the dark.

The heat stimulation of the various areas was carried out under subdued illumination. A 4 watt flourescent bulb was placed outside the experimental chamber slightly above the fish; it was kept on throughout the tests. The light emitted was sufficient to allow the experimenter to see in the mirror where the fish was touched but the fish presumably could not see the experimenter in the darkened room. Location of touch, temperature of probe and particulars about the behavior of the fish during the experiments were recorded on data sheets with fish outlines traced on them.

A foot operated buzzer from a doorbell was secured to the outside of the aquarium. It was to be used for a part of the training procedure.

2. *Training procedure*. A series of first order conditioned reflexes were used during training with the exception of one step that involved second order conditioning (table 1).

Initially the fish was trained to associate bright light with electric shock from which it found relief only in the test box. It could enter from the right or left so that eventually both sides of the fish were tested. As soon as the fish had entered the box, the overhead lights were turned out. At first, sliding doors confined the fish but soon it oriented itself on the door posts, even with the door open, and did not leave the box. As the next step in the training, the fish was touched very gently by advancing the unheated ni-

TABLE 1

TRAINING PROCEDURE FOR MAPPING OF HEAT RECEPTORS

Stimulus	Animal Response
Light + Shock	into test box
Light	into text box
Touch	until no reaction
Heat (38–45 °C)	out of test box
Touch	no reaction
Buzzer and Shock	out of test box
Buzzer	out of text box
Touch	still no reaction
Heat (1–4 °C) + Buzzer	out of box or withdrawal of fin
Heat (1–4 °C)	out of box or withdrawal of fin
(No response below ± 2 °C)	

chrome wire points toward it until it became used to the process and occasionally even leaned against the probes.

Egress from the test box was initially brought about by touch with a probe heated to register 38 to 45 °C when submersed. In each trial, heat was applied only after one, two, three or four, and sometimes even five individual touches in random sequence so that the fish would not expect heat to follow touch after one probing. The rapid exit of the fish from the box at these heated touches lead one to believe that this level of temperature stimulation is noxious.

The fish were given a reward of three to five minutes rest outside the test box before the light was turned on again. In the next step of the training procedure, they were taught to associate the sound of the buzzer with an electric shock which served to drive them out of the test box. The habituation to unheated touch was, however, maintained during this part of the training until, (a) the fish always moved into the box on the bright light, (b) did not react to touches, and (c) would leave the box on the sound of the buzzer.

Only after the fish had learned these three different tasks and performed them well was unheated touch at times alternated with touch of a slightly heated probe. The temperature of this low heat stimulation varied from one to four degrees C. above that of the surrounding water which in turn fluctuated between 18 and 20 degrees during the course of the experiment. Since the fish were continuously kept in the same room they were acclimated to that temperature range and the water temperature was thus constant for any set of trials.

When the buzzer was sounded immediately following the gentle heat stimulation, it was expected that the fish would be able to extend this association to touch of low heat by a second order conditioned reflex from its recent training. Thus it was expected to associate buzzer with shock. If the animal could learn this, its response or lack thereof might give a clue of the threshold of heat stimulation for a small area on the surface of its body. Perhaps very few or even only one individual receptor unit could thus be made to respond. The results to be presently discussed below support this assumption.

The overall heat sensitivity of goldfish was ascertained in a separate set of experiments by a modification of Dijkgraaf's and Bull's (loc. cit.) techniques.

An important feature of these experiments, which will be reported in a separate paper, was the inclusion of one randomly chosen control among six fish at each trial.

Many observations of the control fish established that there was no extraneous clue and that the fish under test treatment always reacted to warm upwelling currents they had been taught to associate with a food reward.

The smallest heat increment reacted to was less than .1°C over a fraction of a second. The animals used for these tests were somewhat smaller than those used for spot stimulation, uniform in size, measuring about 11 cm. total length.

3. *The experimental animals.* Goldfish were chosen because of the ease in keeping and handling them. In the study on point heat stimulation, six animals were trained; they varied in size from eight to 18 cm total length and as far as could be ascertained from the scales were from two to eight years old. Complete training of a fish required up to a hundred trials and only five to seven of those could be performed per day. When more were attempted the animals became refractory. After training, 50 to 80 test trials were made, each again to be spaced not to exceed five to seven a day. Thus only three animals were put through the entire testing procedure, and it is on their performance that the following discussion is in the main based. The behavior of these few animals was so consistent that additional trials could hardly be expected to strengthen the evidence.

RESULTS AND DISCUSSION

1. The fish were capable of forming the reflex associations required of them and their behavior on heat stimulation of all intensities was sufficiently distinctive to conclude that they were reacting to heat and not to touch. The difficulties encountered in the training make it appear likely, however, that several integrated first order conditioning processes and one second order conditioning represent a fish's limits of learning, at least in an artificial situation such as the one I employed.

2. Once the training had progressed sufficiently to work with the probes that were heated to four degrees or less over the surrounding water, the fish sometimes reacted to the warm touch on the fins with a withdrawal of the fin followed by a slight tremor. This, again, was never elicited by touch alone.

3. The smallest heat increment reacted to when applied gently but instantaneously on one area of 2 sq. mm was ± 2°C. Heat sensitivity appeared to exist on the entire fish including the base, the surface, and some spots on the fringes of all fins.

No differences in sensitivity on any particular part of the body could be detected during the course of these experiments. It is possible that such differentiation exists and that it could be demonstrated with a different or more refined method.

4. In 17 out of 150 stimulations the fish clearly gave no response to low heat between 2° and 4°C applied on their bodies; in an additional 13 trials the response was doubtful with eight of these doubtful responses occurring on the fringes of the fins while the rest was observed on the back and the posterior part of the head. In these cases the fish could be made to leave the experimental chamber only by substantial heat increments applied to the same area. Such "blank spots" on the body were less numerous than the areas on which low heat was perceived. They may have contained fewer temperature receptors than their surroundings or, and this is more likely, the failure of the fish to react was due to behavioral irregularities, to be expected in experiments of this kind. In the stimulation of the head region, I experienced considerable difficulty, probably because of the wider spread lateral line system which may have reacted to even the gentlest approach of probes and because the probes were moving close to the eye.

5. Although the heat sensitivity of the entire animal has already been mentioned under the training procedure, it should be emphasized that the fish reacted to a rapid change of ±0.1°C carried by convection currents. Slow and gradual changes of .2 degrees spread over two hours were ignored by the trained animals when they were selected as controls.

A low threshold in perception when temperature changes could be felt over the entire body surface, presumably exciting many or all receptors compared to a very much higher threshold of response to point heat stimulation is the result of areal summation in the performance of individual warm sensitive nerve endings in the skin. According to Krause's (loc. cit.) histological investigations there are in an area of about $\frac{1}{8}$ of a square millimeter of a pike's skin, 7–10 free nerve endings derived from 2 or 3 fibers. None of these are detectably specialized and some out of this profusion (considering the entire surface of the fish) are probably warm or cold receptors while others react to touch. My experiments indicate that there are a few thousand unit warm receptors distributed over the body surface of the goldfish.

Before using the thermode of ± 2 mm^2, a pinpoint heater was tried. It had to be heated to register 10–15°C above the surrounding water, often reaching a noxious temperature level (see above) before the animal responded and frequently only after being touched for several seconds. The 2 mm^2 thermode, however, at 2°C and above brought reproducible and immediate responses. This suggests that the area subserved by a unit receptor—whatever the number of separate endings involved—is around 2 mm^2. It may be somewhat larger still if occurrence of "blank spots" (noted in 4 above) is taken into consideration.

In that case, one of our fish whose surface area on one side was 122 cm^2 might have around 6000 heat-sensitive spots on that side (blank areas were disregarded in this estimate).

The chance that warmth was registered even when the area adjacent to one or between several heat receptors was stimulated might tend to make this estimate too high; it is, however, safe to assume that a few thousand unit receptors are involved. It is probably a coincidence that this number is comparable to the total number of individual heat receptors estimated to exist in the rattlesnake pit organ (Bullock and Faulstick, 1953). Even though the number of nerve units involved may be comparable it should be mentioned that they are diffuse in the fish and concentrated in the pit of the rattlesnake. This arrangement as well as the possible amplification by accessory structures of the pit organ presumably facilitate central reactions to changes in the discharge rates of the nerves and explain the high heat discriminatory capacity of the snake.

In the human skin also the principle of areal summation has been demonstrated by Hardy and Oppel (loc. cit.). Here several hundred warm spots, when reacting simultaneously in an area of 200 cm^2 on the more heat sensitive areas of the body surface were capable of distinguishing much finer changes in heat energy flow than the goldfish and some other fishes which have been investigated (Bull, loc. cit.).

In connection with the 2°C found here as threshold stimulus on a small area, it may be interesting to note that Bohnenkamp and Schroer (1932) reported radiation of about 1.6°C to be the threshold for one warm sensitive spot on the forearm, while Bazzett and McGlone report .3°C at a rate of .2°C per second to suffice as stimulation on a single warm spot on the human prepuce.

The temperature sensitivity of Elasmobranchs which relies on the change in spontaneous discharge rates of the ampullae of Lorenzini has not yet been shown directly to rely on areal summation though the circumstantial evidence for this could hardly be more convincing (Sand, loc. cit.). Recently Hensel (1955) has extended Sand's observations by using a cathode ray oscilloscope.

Bullock (1954), stresses the fact that in every known case of detection of fine and rapid temperature changes areal summation of many—up to several thousand—receptor or receptor-small-fiber units seems to be involved and that, with few exceptions, 2°C seems to be close to the threshold for single receptors.

The secondary anatomy of the facial pit of a rattlesnake (Bullock, loc. cit.) endowed the animal not only with extreme sensitivity but also enables its owner to sense direction of the impinging change in heat flow. It is radiation rather than convection that the snake reacts to but if one considers the relative importance of convection in water and the fact that a fish's prey is almost always in thermal equilibrium with the surroundings it becomes unlikely that a set of specialized directional heat analyzers should have evolved in fishes.

Lastly, it should be stressed that the temperature receptor systems cited for comparison have been shown to involve spontaneous nervous discharge, with discharge frequencies changing drastically upon fast heat changes in the environment.

I have not yet investigated an isolated skin nerve preparation but on the evidence accumulated so far it seems reasonable to expect to find another instance of a continuously discharging system. The individual receptor or the receptor-small-fiber-unit only produces "noticeable" or sufficiently significant changes at relatively large changes in environmental temperature. As more units become involved, smaller changes at slower rates are reacted to.

The cautions advocated by Hensel (1952) on account of the central thresholds for temperature sensations apply to my experiments. For this reason it should be attempted to isolate a skin-nerve preparation in order to gain knowledge on the performance of the receptors themselves. Yet information on what constitutes the adequate stimulus for the functional combination of peripheral and central elements can only come from the whole animal.

SUMMARY

By conditioned reflex training it was found that goldfish sense ambient temperature increments of about .1°C over their entire body surface. Even the fins bear warm sensitive nerve endings. The receptors apparently operate on the principle of areal summation with an instantaneous heat increment of 2°C as the lower limit to which a fish would react when an area of 2 sq. mm was stimulated with heated probes after it had been trained not to react to touch stimuli.

An estimate of the total number of units involved in relative temperature discrimination suggested that several thousand units are involved; this number is of a comparable order of magnitude to that found in other vertebrates with a similarly fine or finer level of heat discriminatory capacity. This order of magnitude of unit receptors is found, be they collected in special organs or scattered over the entire body surface.

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THE INFLUENCE OF BREEDING HABIT ON THE OUTCOME OF NATURAL HYBRIDIZATION IN PLANTS[1]

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Introgression and polyploidy are the two most common results of natural interspecific hybridization in plants. A great deal of knowledge has been gained about each one of these phenomena. The controlling factors which determine whether a given natural hybrid will backcross to the parental species or give rise to allopolyploid derivatives have not, however, been clearly defined. The purpose of the following paragraphs is to record some observations on the relation between the life cycle and breeding behavior of the parental species and the course of developments subsequent to the formation of a natural hybrid.

With the exception of a single race of one species, every polyploid in Gilia subgenus Gilia (Polemoniaceae) is small-flowered and self-pollinating (Grant, 1953; Grant, Beeks and Latimer, 1956). A correlation between self-pollinating habit and polyploid constitution is found also in the tribe Madiinae (Compositae) and the genus Microseris (Compositae-Cichorieae), (Clausen, Keck and Hiesey, 1945; Chambers, 1955). If the small size of the flowers is a criterion of autogamy, the same correlation is exhibited in Mentzelia (Loasaceae), Escholtzia (Papaveraceae), and Clarkia (Onagraceae) (Thompson and Lewis, 1955; Lewis and Snow, 1951; Lewis and Lewis, 1955).

A feature common to the aforementioned phylads, or at least to the sections of them including the polyploids, is the possession of an annual life cycle. The tendency for polyploidy to become established more frequently in selfers than in outcrossers in annual groups may be related to the mode of origin of the polyploid condition.

The most common process by which the chromosome number is doubled in annual plants seems to be non-reduction in natural hybrids (Clausen, Keck and Hiesey, 1945). The chances of a union of two unreduced gametes produced by a diploid hybrid will be most favorable if the plant reproduces by self-pollination. A hybrid which is committed by the characteristics inherited from the parental species to a system of outcrossing might not be able to give rise to any polyploid derivatives in the space of a single short blooming season. Another hybrid capable of self-pollination might produce one or several polyploid zygotes in the same period of time.

Where the time available for reproduction by the hybrid is critical, as in an annual herb, the breeding behavior of the parental species will therefore operate as a selective mechanism controlling the origin of polyploids.

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[1] This paper has resulted from a program of research aided by a grant from the National Science Foundation.

This mechanism will favor the formation of polyploid types under conditions of self-pollination, but not under conditions of outcrossing, and will thus lead to the correlation observed in several unrelated groups of annuals between high chromosome numbers and small size of flowers.

Natural hybrids are no doubt formed between species representing every type of breeding system. The outcome of the hybridization may differ, however, according to the breeding behavior of the parental species. The large-flowered outcrossing species of Gilia subg. Gilia are, with but a single exception (*Gilia ophthalmoides flavocincta*), diploid; and a majority of these species are affected by introgression. The small-flowered self-pollinating diploid species, on the other hand, are relatively uncontaminated by introgression, but have been the starting points of the various polyploid species. The hypothesis may be considered, therefore, that the result of natural hybridization in annual plant groups will tend to be introgression when this hybridization involves cross-fertilizing species, and polyploidy when it involves self-fertilizing species.

It is well known that the conditions for establishment of a polyploid condition are generally more favorable in perennial herbs than in annuals (Müntzing, 1935; Stebbins, 1938, 1950, ch. 9; Gustafsson, 1947, 1948). The time available for a union of unreduced gametes is longer in the perennial types and the process of somatic doubling is more apt to take place. The hybrid *Primula verticillata* × *floribunda*, to cite a classical example, did not give rise to the tetraploid, *Primula kewensis*, in the first year of its existence, and it is fair to assume that the polyploid might never have originated if the parental species had been annuals. These considerations account for the more frequent occurrence of polyploidy in perennial herbs than in annuals.

Biennials, monocarpic perennials, and other short-lived perennial herbs without effective means of vegetative propagation frequently exhibit less polyploidy than the most closely related groups of annuals. This is the case in comparisons of the biennial Gilias and Ipomopsises with the annual Gilias, the biennial with the annual sections of Mentzelia, and the perennial with the annual sections of Microseris (Grant, 1956; Thompson and Lewis, 1955; Chambers, 1955). Polyploidy is either absent or very rare in the short-lived perennial groups, but is fairly common in the related annual forms.

In Ipomopsis (Polemoniaceae) the cross-fertilizing biennial groups are apparently entirely diploid, whereas tetraploidy is known in the outcrossing perennial *I. multiflora*. Similarly in Gilia, polyploidy is found in the autogamous annuals, as already noted, and also in the cross-fertilizing perennial group centering on *Gilia rigidula*, but is unknown in the cross-fertilizing biennial *Gilia stenothyrsa* group (Grant, unpubl.).

The explanation of the relatively low frequency of polyploidy in the biennial and short-lived perennial herbs mentioned above may lie in their habit of maintaining a high level of outcrossing. In the perennial species of Ipomopsis and Microseris this outcrossing is promoted by self-incompatibility, and in the biennial Mentzelias by the floral mechanism. The annual relatives of these groups are predominantly self-compatible and undergo considerable self-fertilization under natural conditions. A life cycle which is long enough to tolerate the luxury of obligate cross-fertilization, but which is too short to afford much scope for somatic doubling or the chance union of unreduced gametes produced by different individuals, evidently presents very unfavorable conditions for the appearance of polyploidy.

Narural hybridization between self-incompatible and highly outcrossing species of biennial and short-lived perennial herbs will thus be expected to lead most frequently to introgression and only rarely to the formation of new polyploid forms. This expectation is fully realized in the biennial sections of Ipomopsis, the species of which are extensively affected by introgression on the diploid level. Short-lived perennials with an autogamous breeding system, on the other hand, may be expected to give rise to polyploid derivatives of hybrids at least as frequently as autogamous annuals.

Still another outcome of natural hybridization, apomixis, is confined, so far as is known, to long-lived herbaceous and woody groups which are regularly cross-fertilized in consequence of self-incompatibility or dioecism (Gustafsson, 1948; Stebbins, 1950, ch. 10). Polyploidy and introgression also occur frequently in perennial herbs and woody plants with a cross-fertilizing habit of breeding. A variety of courses of development is thus open to a long-lived, vegetatively reproducing hybrid with an allogamous breeding system.

SUMMARY

Where the time available for reproduction by a natural hybrid is critical, as it is in annual herbs, biennials, and short-lived perennials, the natural hybridization will tend to run toward polyploidy under conditions of self-fertilization and toward introgression under conditions of outcrossing. We accordingly find in some annual and biennial groups, where hybridization has occurred on an extensive scale, that polyploidy is closely associated with autogamy and introgression with outcrossing. In long-lived perennial plants these tendencies may be obscured and reversed by other factors. The association of allopolyploidy with an outcrossing breeding system, which was noted by Gustafsson (1947), is found chiefly in plants with a perennial habit and/or vegetative means of propagation.

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FURTHER STUDIES ON THE REVERSAL IN THE SEEDLING HEIGHT DOSE CURVE AT VERY HIGH LEVELS OF IONIZING RADIATIONS

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In an earlier publication (Schwartz, 1954), a preliminary report was presented describing the effect of high doses of ionizing radiations on the germination and growth of maize seeds. The dose curves, plotting seedling height against dose, indicated that, whereas at the low dose levels there is the expected inverse relation, a point is reached after which the original trend is reversed and a further increase in radiation dose results in an increase in seedling height. The low point in growth occurs at approximately 125,000 r of gamma rays. A typical dose curve is shown in Fig. 1. The purpose of this paper is to present an hypothesis to explain this phenomenon and the results of experiments designed to test its validity.

In the earlier studies it was noted that, at the high dose levels, around 500,000 r, where the seedlings were turgid and looked perfectly normal, growth had ceased after about five days. Root-tip smears showed no division figures, which suggested that the growth of these seedlings was due entirely to the elongation of those cells already present in the embryo at the time of irradiation.

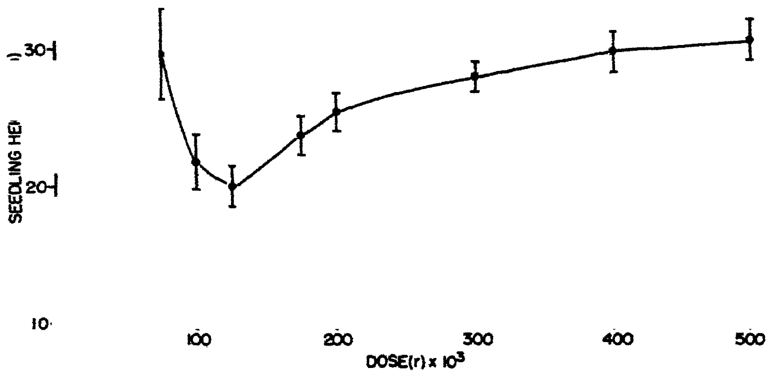

FIGURE 1. Relation between seedling height and gamma dose from Co^{60} source. Confidence limits are 95 per cent. (From Schwartz, 1954.)

It is well established that there is a direct relation between dose of ionizing radiation and chromosome breakage; it is also known that ionizing

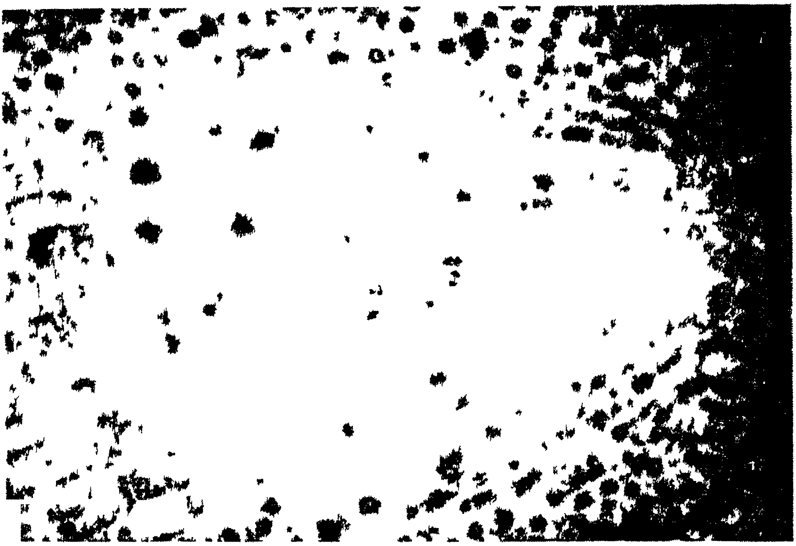

FIGURE 2. Longitudinal section through a young root tip from seed irradiated at 120,000 r of gamma rays.

radiations have an inhibitory effect on cell division (see Lea, 1947). Thus, with increased dose, as the average number of chromosome breaks per cell increases, the probability of mitosis is diminished. The genetic complement of a cell in which a large number of chromosome breaks have been induced will remain intact as long as the cell does not divide. Distribution

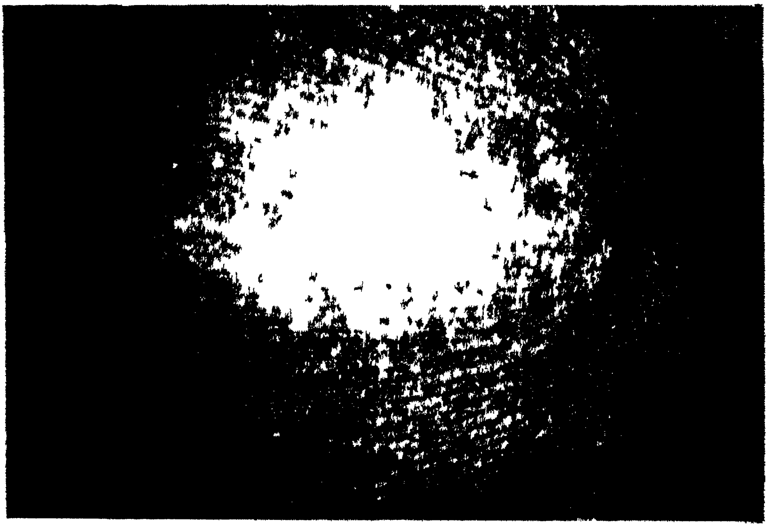

FIGURE 3. Section through young root from 500,000-r material showing complete absence of mitotic figures.

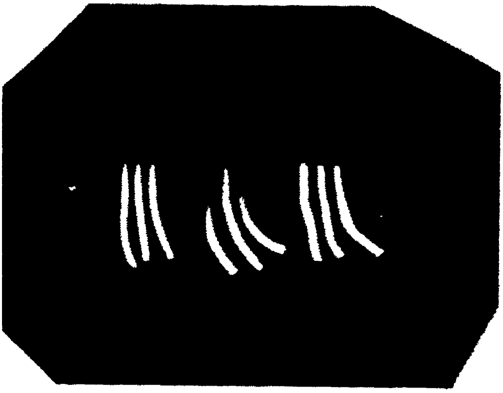

FIGURE 4. Root tips (tip up) from older seedlings with the following treatments: Left, control; middle, 120,000 r; and right, 500,000 r. The necrosis which is evident at 120,000 r does not appear in the 500,000-r material.

of its genes on many fragments rather than on whole chromosomes would not be expected to impair their function. However, because of aneuploidy, division will very likely result in death of the daughter cells. It is therefore proposed that, at the very high doses, the seedlings grow to their maximum height by elongation alone, with division being completely inhibited. As the dose is reduced to around 125,000 r of gamma rays, there is a de-

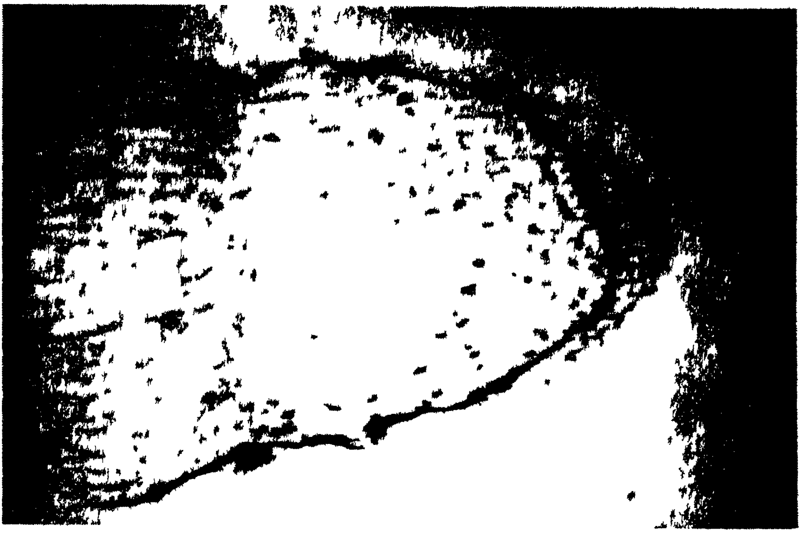

FIGURE 5. Section through older seedling root tip from the 500,000-r material. The cells at the tip have elongated and lost their meristamatic appearance.

crease in chromosome breakage, but an increase in cell lethality, since some of the damaged cells are now able to divide. As the dose is further reduced below 125,000 r, chromosome breakage is reduced to such an extent that even though the cells divide, lethality does not result and there is a marked increase in seedling growth. Cell division in the maize seedling is almost entirely confined to the region just below the coleoptilar node. It is to be expected that cell lethality in this region through which water and nutrients must travel to the plumule would have a drastic and very noticeable effect on the elongation and viability of cells in the higher regions.

On the basis of this hypothesis, certain predictions can be used to test its validity. The seedlings in the 125,000-r dose range, where the least growth is made, should show many more division figures than the taller, healthy-looking material at the higher doses. Also, a large amount of necrosis should occur in tissue in the region of cell division for the 125,000-r range seedlings and not at the higher doses. Both these predictions are borne out. There are still a fair number of cells in mitosis at 120,000 r, approximately one-third the number found in the control. Much chromosome damage, detected as bridges and fragments at anaphase, is evident at these doses (Fig. 2). No division figures were found in material that received doses in excess of 400,000 r (Fig. 3). The root tips used for these studies were removed from young seedlings whose roots had attained a length of approximately 1 cm and had not as yet shwon any signs of necrosis. Other seedlings were allowed to attain their maximum growth and were then examined for signs of cell lethality. Necrosis was very evident around the 125,000-r range, with none observed in the controls and at very high doses (Fig. 4).

The appearance of the root tips at the very high doses is worthy of some comment. Although they showed no sign of lethality, they were nevertheless quite abnormal in appearance, square rather than pointed and looked very much as though the root had been severed some distance above the tip. The cause of this abnormal appearance became apparent when these roots were sectioned and observed cytologically. Unable to divide, the cells in the tip region underwent elongation and maturation in spite of the large degree of chromosome breakage (Fig. 5) and lost their meristematic appearance.

SUMMARY

The growth of seedlings irradiated as seeds with high doses is shown to be dependent on two main factors—chromosomal damage and rate of mitosis. The interaction of these factors is responsible for the reversal of the dose curve where seedling height is plotted against dose. At the very high doses, a point is reached where the effect of increased chromosomal damage is masked by the cessation of cell division.

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LETTERS TO THE EDITORS

Correspondents alone are responsible for statements and opinions expressed. Letters are dated when received in the editorial office.

A SECOND CASE OF TRANSLOCATION BETWEEN PATERNAL AND MATERNAL CHROMOSOMES*

Following X-ray treatment of adult *Drosophila melanogaster* males Sidky (1940) observed an unusual segregation of characters which he interpreted as the result of a translocation occurring during an early embryonic stage between the third chromosome derived from an irradiated sperm and the Y chromosome derived from an untreated egg of the attached-X mother. Although the evidence appeared to be decisive the interpretation has recently been questioned (Glass, 1955). Recently, during the course of an experiment in which translocations induced in mature sperm were detected, further evidence has been obtained to support Sidky's contention that rearrangement between paternal and maternal chromosomes can occur at an embryonic stage prior to polar cap formation.

Males of the "multipurpose stock" (Muller, 1954), sc^8.Y/y In49 *B*; bw^D, were mated to "multi-females", X.Y InEN *y*; *st*, and a 0.25% solution of a nitrogen mustard in 0.4% NaCl solution was administered to inseminated females as a vaginal douche. Translocations were detected by crossing F_1 males individually to virgin females like their mothers and the presence of a translocation among the Y, second and third chromosomes was indicated by the absence of recombinant classes involving the markers y, bw^D and *st.*

In a single F_2 culture the following classes of offspring were obtained: $y^+bw^+st^+$ 19 ♂♂, $y\ bw^+\ st^+$ 8 ♀♀, y^+bw^Dst 16 ♂♂ and $y\ bw^Dst$ 11 ♀♀. The parental male was recovered and mated to three more multi-females. Again only the same four classes of offspring, with the exception of one non-disjunctionally-produced $y\ bw^D\ st$ ♂, were observed in a total of 165 flies. It appeared that a translocation between paternal and maternal second and third chromosomes had taken place. In order to determine whether the rearrangement involved the paternal second chromosome and the maternal third chromosome or vice versa the following crosses were made: (1) F_2 males phenotypically y^+bw^Dst, with the marker bw^D which was derived from the original P_1 paternal second chromosome, and one of the st markers which was derived from the original P_1 maternal third chromosome, were mated to wild-type Oregon-R virgin females. If these males carried the translocated chromosomes, the latter would have been distributed to the F_3 offspring which were phenotypically bw^D and upon subsequent crossing of these F_3 males to multi-females only the same four types of offspring as were observed in the F_2 would have appeared in the F_4 generation. (2) F_2 males phenotypically $y^+bw^+st^+$, with one of the unmarked second chromosomes which was derived from the original P_1 maternal second chromosome, and the st^+ third chromosome which was derived from the original P_1 paternal chromosome, were mated to virgin females with the genotype y/y; $^+/bw^D$ st/st. If now these males carried the translocated chromosomes, the latter would have been distributed to the F_3 offspring which were phenotypically bw^Dst^+ and upon subsequent crossing of these F_3 males to multi-females only the same four classes of offspring as were observed in the F_2 would have appeared in the F_4 generation.

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*This work has been supported by a grant received for work of H. J. Muller and associates from the American Cancer Society, on recommendation of the Committee on Growth of the National Research Council. Contribution 606, Department of Zoology, Indiana University.

When these two sets of crosses were made, F_4 cultures derived from F_2 y^+bw^Dst males yielded the same four classes of offspring as noted in the F_2 while the F_4 cultures derived from F_2 $y^+bw^+st^+$ males yielded all recombinant types. Therefore the original translocation must have been either a reciprocal interchange between the paternal second chromosome and the maternal third chromosome or, less likely, since it would have required three breaks, a rearrangement of the deletion-insertion type between these chromosomes.

Most simply it could be assumed that a break induced in a sperm chromosome remained open until after fertilization at which time the broken pieces underwent interchange with induced or spontaneously broken pieces of an egg chromosome. With respect to mature sperm, in inseminated females at least, the evidence (Muller, 1940) is clear that breaks remain open until after fertilization. The evidence concerning breakage and rearrangements in oöcytes reveals a different situation. The main basis for his skepticism concerning Sidky's interpretation was the rarity with which translocations in females were found to occur, even following irradiation, in Glass's own work. But as Sidky was careful to point out, the occurrence of single chromosome breaks, spontaneous as well as induced, must be far more frequent than the recoverable eucentric interchanges which require the coincidence of two breaks and reunions. Furthermore, recent evidence (Herskowitz and Muller, 1953; Muller and Herskowitz, 1954; Herskowitz and Abrahamson, 1955) indicates that certain kinds of multi-break rearrangements, half-translocations, can be induced in females with a high frequency, and in addition breaks can rejoin prior to fertilization. With this latter point in mind it should be noted that in the present case the assumption is just as probable that even the initial breakage took place within the zygote, since the timing of chemically induced breaks is not limited to the period of external application of the agent. Whether the reason be the length of time necessary for mutagenically active particles to reach sites of breakage in the chromosomes or a "delayed-action", involving some sequence of chemical reactions that lead up to the linkages, it is possible for the entire sequence of breakage and reunion events to be delayed until a number of nuclear divisions after fertilization.

Consequently, unlike Sidky's original translocation between sperm and egg chromosomes, the present case cannot be cited as additional supporting evidence for the firmly established mechanism of interchange, viz., breakage first followed by reunion, or for delay until after fertilization of the union of pieces broken in mature sperm, but it does provide another example of a translocation between paternal and maternal chromosomes. However, both these cases do show that the paternal and maternal sets of chromosomes of Drosophila can become intermingled at a rather early stage, without that strict mutual isolation or "gonomery" which has sometimes been thought to occur.

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PROBLEMS OF SEX IN UNICELLULAR ALGAE

In a recent paper published in German (1955a) and English (1955b), Hartmann has criticised an article of mine (Lewin, 1954), and I feel that a reply is called for.

Among the features which Hartmann seeks to criticise is the "partial" nature of my presentation. However, I did not set out "to give a correct critique of positive and negative findings concerning bipolar bisexuality," etc. My contribution to the 1951 symposium on "Sex in Micro-organisms" was limited to the subject of sex in unicellular algae, and I considered that it was outside my province to discuss other organisms. Nor did I aim to write a comprehensive review, but rather to concentrate on "studies of cultures and experiments under controlled conditions, where a measure of reproducibility can be expected in the results" (Lewin, 1954).

If one of Hartmann's basic theses is that physiological differentiation is an *essential* condition for syngamy, then I feel I must continue to disagree. I know of no unequivocal evidence for intraclonal sex differentiation in any homothallic (haplomonoecious) unicellular alga, although Lerche's unpublished experiments with *Haematococcus* appear indicative. Even if such a condition were confirmed in one case, it might be rash to generalize.

I would prefer not to engage in polemics on priorities or on terminology. The terms "homothallism" and "heterothallism," as defined (with no claim for originality) in my article (p. 103), are in fairly general use in English-speaking countries (c.f. Whitehouse, 1949).

Finally, I might point out that an incomplete quotation from my article (p. 116: c.f. Hartmann, 1955a, p. 328; 1955b, p. 340) distorts the original meaning: and that, in his discussion of sex substances, Hartmann (1955a, pp. 326–328; 1955b, pp. 338–340) appears to have confused sex-substance activity of types 2 and 3. I make these corrections merely in the hope that further misunderstandings may not creep into the literature.

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It is good to have a reappraisal of our taxonomic group's early beginnings by this distinguished student of the protochordates. The author feels that the evidence suggests that it all started with neotenous ascidian larvae that invaded fresh water habitats. These gave rise to an unarmoured type of ostracoderm. Amphioxus is regarded as a degenerate descendant of the first chordates that is "not even a satisfactory vertebrate prototype." The hemichordates are 'not in any direct way relevant to the story.'

J. A. MOORE

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Landblom, Nellie Thompson, 1955. Nellie Landblom's copybook for beginners in research work. 118 p. $2.95 (multigraph). Colorado A. & M. College, Fort Collins, Colorado.

Lees, A. D. 1955. The physiology of diapause in arthropods. 151 p., ill. Cambridge Monographs in Experimental Biology, Vo. 4. University Press, Cambridge.

Diapause is defined as a state of arrested growth other than a mere quiescence brought on directly by an environmental factor. It may occur at any of the major stages of the life history. The second chapter is devoted to a consideration of the influence of environmental factors, notably photoperiod, temperature, and food supply on the onset of diapause whereas the role of water is relegated to a subsequent chapter. Experiment shows that even in insects with obligatory diapause manipulation of the environment may affect the onset of the condition, and hence the occurrence of diapause is subject to selection. Of the environmental factors that terminate diapause temperature is the most important. The life cycles of parasitic insects are synchronized with those of their hosts, contain a period of diapause if this is present in the host, and also show adaptation to host races that differ as regards diapause. Available data on metabolism during diapause are discussed and there is also a chapter on hormonal control of diapause, with adoption of the schemes made familiar by the work of Williams and Wiggleworth. A final chapter discusses diapause as an adaptation to the environmental conditions to which an arthropod is exposed during its usual life cycle.

The book is essentially a review of the literature and as such is a valuable aid but in general lacks synthesis.

L. H. HYMAN

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WHAT IS AN ADAPTIVE TRAIT?*

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MORPHOLOGICAL CONSERVATISM IN SIBLING SPECIES

The idea of the present article, which attempts to deal with the general problem of the nature of adaptedness of traits which differentiate races, species, and other groups of animals and plants, has been suggested by consideration of a topic apparently not closely related. The genus Drosophila contains a number of pairs, or of groups of several, sibling species. This phenomenon is particularly common among Drosophila of Brazil and of the American tropics in general. Sibling species are morphologically so similar, or nearly identical, that distinguishing them by inspection is difficult or impossible. Yet, they are demonstrably good biological species, since the reproductive isolation between them is complete or nearly so. Why is the genetic differentiation in sibling species not accompanied by morphological divergence?

The quadruplet of sibling species, *Drosophila willistoni, D. paulistorum, D. tropicalis,* and *D. equinoxialis,* may be considered here as an example. Sexual isolation between them is so strong that cross-insemination is rare even when no choice of mates is available; when interspecific copulation does occur no viable progeny is produced. The four species are sympatric over extensive territories in South and Central America; the populations of each species contain arrays of chromosomal variants of the inversion type, but there is no evidence of either regular or occasional transfer of these variants from species to species, as would be expected if effective hybridization occurred, however rarely, in natural habitats. Morphological differences between these species exist, especially in the male genitalia, but they are so slight that it is not feasible to distinguish the species by inspection of living individuals under a binocular microscope, at least not in the female sex (Burla et al. 1949, Spassky unpublished). Analysis of the salivary gland chromosomes shows that the gene arrangements in the four species have diverged, especially in one of the chromosomes, the third, which in three of the four species is built so differently that homologous regions are no longer recognizable. There is also good evidence of physiological and ecological divergences. Every species has a distribution area different from the others, as well as habitat preferences of its own. Where the siblings are sympatric they may vary in relative abundance at different seasons. In laboratory cultures, the larval development of *D. willistoni* is faster than that of *D. paulistorum; D. tropicalis* gives beautifully clear polytene chromosomes in the salivary glands, *D. equinoxialis* gives poor chromosomes, while the other two siblings are intermediate.

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*The substance of this article was presented in an address read before the Academia Brasileira de Ciencias, at Rio de Janeiro, on May 30, 1956.

The reasons for the scanty morphological divergence despite considerable genetic and physiological diversification are a matter of speculation. I have surmised that the external morphology of some species groups of Drosophila has reached so high a level of adaptive specialization that most changes in the visible body structures are discouraged by natural selection; nevertheless, room is left for ecological divergence based on physiological differentiation (Dobzhansky 1941).

Attempts to apply this conjecture to concrete cases lead however to difficulties. Indeed, most of the morphological traits which differentiate related species of Drosophila are of a kind the significance of which for the welfare of the animals is not at all evident. A partial list of these traits will make this point clear: Number of branches in the arista, shape of the facial carina, width of the cheek relative to that of the eye, presence of one vs. two prominent bristles in the oral row, thorax more or less polisned or dull, presence or absence and kind of the color pattern on the thorax, number of rows of acrostichal microchaetae on a certain portion of the thorax, anterior scutellar bristles divergent, parallel, or convergent, relative lengths of certain bristles on the pleurae, proportions of certain sections of wing veins, presence or absence and intensity of color markings on the wings, position and kind of bristles on some leg segments, details of the color pattern on the abdomen, shapes of various parts of male genitalia, number of coils in the testes, shape of the spermathecae, number and kind of coils in the ventral seminal receptacle, etc.

ADAPTIVE AND NEUTRAL TRAITS IN CLASSIFICATION

Why should these and other similar traits vary in some species groups of Drosophila, and yet be fixed in species of other groups making the latter sibling species? This problem is only a special case of a more general one, that of the adaptive significance of the keleidoscopic diversity of morphological traits in different organisms. Darwin was quite aware that the morphological differences which systematists use to discriminate between species, subgenera, genera, tribes, families, and other groups often concern surprisingly trivial traits, of no apparent consequence to the organisms involved. It was probably this, more than any other difficulty, which led during the first quarter of the current century to a temporary eclipse of the theory of natural selection. If most differences which we observe between organisms were adaptively neutral, then it would seem that factors other than selection must be invoked to account for the origin and maintenance of these differences.

It has been repeatedly suggested in biological and anthropological literature, even in recent years, that classification of organisms should be based on adaptively neutral traits. Allegedly, the traits which accurately reflect homology and common descent are neutral, while positively adaptive traits are too easily modified by the environment and are apt to show analogy instead of homology, ecological correspondence rather than common descent. But if so, then the evolution of traits on which the classification is based is a mystery. To use Drosophila again as an illustration: the more than 600 known species of this genus all have three orbital bristles on either side of their heads, and the anterior of these bristles is always proclinate (bent forward), while the other two are reclinate (bent backward). Now, why should this character be retained so tenaciously in so many species? Is it really important for the flies of this genus to have one proclinate and two reclinate orbital bristles?

The basic postulate of the modern biological theory of evolution is that adaptation to the environment is the guiding force of evolutionary change. We need not follow the extremists who insist that all evolutionary changes are adaptive; however, we have to suppose that most organs and functions of most organisms are, or at least were at the time when they were formed, in some way useful to their possessors. Nothing less than this is acceptable if the modern theory of evolution is sustained.

The argument is, then, that our failure to see the adaptive singificance of most traits is simply a measure of our ignorance. Some authors have even urged that assuming neutrality of traits is methodologically inadmissible, since such an assumption allegedly prevents making observations and experiments needed to discover their adaptive significance. The work of several investigators (e. g. Lamotte 1951, 1952) has at the very least shown that supposing some traits to be close to adaptive neutrality may be a fruitful working hypothesis. The usefulness of a trait must be demonstrated, it cannot just be taken for granted. All that can be said is that one's failure to see the adaptive significance of a trait is no proof that it has none. The matter must be settled on the basis of evidence, not by theoretical arguments alone.

It is a fact that the variation in some traits which at one time seemed neutral was later discovered to be adaptive. The chromosomal inversions in Drosophila populations are an example (Dobzhansky 1947); the colors and color patterns of Cepaea snails are another (Cain and Sheppard 1954). But it is still as true today as it was in Darwin's day that the adaptive significance of most traits which vary between and within species is obscure. Suffice it to mention that practically nothing is known about the significance of most traits which vary between races of man as well as among individuals within a race. Although Coon (1955) has made some interesting suggestions concerning the possible roles of such apparently neutral human traits as the form of the nose, lips, hair, and similar variations, the matter awaits experimental verification. When one considers traits in which species of insects and other organisms often differ, such as the differences between Drosophila species mentioned above, the supposition that all or even most of them are directly useful to their possessors stretches too much one's credulity.

CORRELATION OF TRAITS

At this point we should inevitably inquire whether the impasse which we seem to have reached in our argument may be due to a faulty statement of the problem. A visible "trait" is an outcome of the occurrence of certain developmental, physiological, and ultimately physico-chemical, processes in the organism. Traits, such as the presence of one proclinate and two reclinate orbital bristles in Drosophila, need not by themselves be of any particular importance to the animal. In fact, some Drosophila mutants have one or more of the orbital bristles missing, and the mutant flies seem to suffer no inconvenience on this account. But the processes which result in the formation of certain bristles may give rise also to the other traits, morphological and physiological, in the same organism. The proclinate or reclinate position of a bristle, though quite unimportant in itself, may be an outward visible sign of the occurrence in the organism of quite important developmental processes. The latter are not necessarily disturbed when other factors cause some particular bristle to be missing; a mutant may survive without it. However, if a bristle is formed at a certain point of the hypoderm it is determined either as proclinate or reclinate (Stern 1954). The fixation in the phylogeny of Drosophila of this and of other apparently equally unimportant traits as generic or specific characteristics is, then, a consequence of the adaptiveness of the processes of which these traits are the concomitants.

It cannot be stressed too often that natural selection does not operate with separate "traits." Selection favors genotypes the carriers of which transmit their genes to succeeding generations more efficiently than do the carriers of other genotypes. Now, the reproductive success of a genotype is determined by the totality of the traits and qualities which it produces in a given environment. A disadvantage in some respects may be compensated by advantages in other respects. Thus, in an experiment of the writer with laboratory populations of Drosophila, a genotype which was weaker than another genotype in larval competition under crowding had nevertheless a higher adaptive value, presumably because of a higher fecundity of adults (see also Birch 1955). When a trait is a part of a complex, a system, or a syndrome of developmentally correlated characters, it is obviously the whole system which is favored or discriminated against by natural selection. This is not contradicted by the fact that some one character may be of paramount significance in deciding the success or failure of a genotype in a given environment and at a given time. Frost resistance may be decisive during hard winters, and drought resistance during dry summers.

PLEIOTROPISM

When genotypes which differ in a single gene yield phenotypes which are unlike each other in two or more not obviously related traits, this gene is said to have pleiotropic, or manifold, effects. Examples of pleiotropism are common. The classical mutants of Drosophila were detected and identified almost always by some externally visible abnormalities in the structures of the adult fly. And yet, these mutants manifest very often also some variations in the viability, fertility, longevity, and/or other physiological characteristics. When such mutants are submitted to the action of natural selection, their fate is likely to be determined not by their obvious externally visible characteristics but by the more subtle physiological ones. Reed and Reed (1950) and Merrell (1953) found that the white-eyed mutant of *Drosophila melanogaster* is discriminated against by natural selection in certain experimental environments; however, this occurs not because their pigmentless eyes make the white flies blind, but chiefly because the females, both red-eyed and white-eyed ones, accept the advances of normal red-eyed males more often than they do those of the white-eyed males.

Quite trivial, and by themselves adaptively neutral traits, may then, be established or eliminated by natural selection because they are components of genetic syndromes which contain also some selectively effective features. It is unimportant in this connection whether the different parts of a syndrome are due to a multiple "primary" action of a gene or to branching of a single "primary" action in the development. This distinction (Grüneberg 1938) has operationally little meaning at the present level of our knowledge. Whether or not genes produce different "primary" products in the same or in different tissues at different stages of the development is an intractable problem which has not been solved. Regardless of what may be its eventual solution, the characteristics of animals or plants which appear striking to our eyes are often less important to their possessors in the struggle for life than the less obvious genetic and developmental correlates of these characteristics. In looking for an explanation of the evolutionary behavior of a trait the adaptive significance of correlated traits cannot be ignored.

The evolutionary importance of pleiotropism is not lessened by the fact that most mutations of single genes are reflected on the phenotypic level by altering some one trait apparently more strongly than others. For example, known mutations at the white locus in Drosophila all affect the eye color, no matter what other characters they may influence at the same time. White is supposed to be an "eye-color gene." In microorganisms, mutations which are characterized biochemically usually affect one and only one step in some reaction chain. But it should not be forgotten as it often is, that by comparing phenotypic effects of gene alleles A_1 and A_2 we learn at most which developmental processes are influenced by the *difference* between these alleles. We still do not know the total extent of the field of action of either A_1 and A_2. To find this out, it is necessary to compare the effects of the absence (deficiency) of the gene A with those of its presence. But even when this is possible, one cannot be sure that genes homologous to A are not carried in the same genotype at other loci. Recent work on pseudoalleles has indeed shown that gene duplication occurs in the evolutionary process more often than previously known. One cannot exclude the possibility that the presence in the genotype of at least one unit of every kind of gene which a species possesses may be essential for the life of the carriers of this genotype.

GENES AND DEVELOPMENTAL SYSTEMS

Pleiotropism is clearly a part of the story of the establishment in evolution of apparently useless traits, but it can hardly be the whole story. The problem is really insoluble as long as we think of every gene as controlling one or more traits or processes in isolation from the development of the organism as a whole. What, indeed, are the particular adaptively important traits which are correlated with the presence of one proclinate and two reclinate oral bristles in Drosophila? Perhaps such traits will some day be discovered, but at present it may be useful to explore the possibilities of other and more general solutions.

The development of an individual spans the interval of time which begins at fertilization of the ovum and concludes at death. The sequence of events which occur during this time is described most easily in terms of the appearance, persistence, and disappearance of various "traits" and "processes." The same is true of phylogenetic development, except that at least the beginning of the sequence of the events is here always lost in the dimness of the past. For example, we do know, or reasonably infer, that in the human ancestry the trait "erect posture" appeared, and the trait "facial prognathism" disappeared, at a certain time.

In so describing our observations on the ontogeny or the phylogeny one should, however, keep in mind that the individual and phylogenetic developments do not consist of a series of discrete events but are really continua in time. The isolation of "traits" is, then, only a semantic device valid and useful in recording our observations. However, the usefulness and validity of this device lapse as soon as the "traits" begin to be thought of as having independent existence. But this is precisely what all too often happens in the thinking of many biologists. Both the ontogeny and the phylogeny are visualized as building a kind of mosaic picture or as putting together a jigsaw puzzle. The stones of the mosaic, or the pieces of the jigsaw puzzle, are the traits, each determined by one or by several genes, which are gradually assembled by accretion until the picture (organism) is complete, or until it reaches the state in which the observer studies it. Now, this is a shockingly unrealistic way of representing either the ontogenetic or the phylogenetic development. It becomes a source of pseudo-problems, one of which may be the apparent prevalence of traits which seem to have no adaptive function to perform in the organisms which possess them.

Here we come upon a paradox no biologist should avoid facing. Among the major achievements of modern biology is the discovery that the heredity which is handed down from parents to offspring is a constellation of self-reproducing corpuscles of molecular dimensions, genes. Yet, the development of individuals, as well as the evolution of Mendelian populations, are highly integrated processes. Even if the genes had each a single primary function, such as the production of a single enzyme (which may well be questioned), it would remain true that their effects are woven together on the developmental level into a single system which undergoes orderly changes in time. Genes do not determine each a separate trait; they determine, jointly and severally, the developmental processes which result in the appearance of all the traits which an organism possesses at all stages of its life cycle.

The fact that the changes known at the locus "white" in Drosophila alter the eye color of the fly does not exclude the possibility that this locus has other functions as well. As stated above, the white mutant indeed shows biotically important characteristics which are not obviously related to the color of its eyes. It is even more misleading to say, as is frequently done, that the eye color is formed by the gene white. Although in order for the pigment to be formed and deposited in the eyes the presence of certain alleles of this gene is necessary, many other genes are known which also influence the formation of the pigment and the development of the eyes. Microcephalic idiocy in man may be due to a mutation in a certain gene, but it does not follow that the human head is formed by that gene. Formation of the head is an outcome of embryonic and post-embryonic development which is influenced by probably all the genes which the organism has, and the presence of most and perhaps of all these genes is indispensable for the development to proceed normally. The genes are to some extent independent of each other in the processes of the transmission of heredity from parents to offspring, even though studies on position effects and on partial allelism suggest that this independence is not as complete as classical geneticists liked to think. By contrast, the processes of development cannot be divided in autonomous components governed by single genes. The verbalisms borrowed from studies on the transmission of heredity are often misleading when applied to the phenomena of development. It is unfortunate that this illegitimate transfer of words, and of habits of thought which go with them, is nevertheless a common practice with geneticists.

On the population level the genes are rarely autonomous. Genes as such are subject to natural selection perhaps only in simplest viruses. Elsewhere it is not a separate gene but the genotype of which it is a part that is favored or discriminated against by selection. Moreover, the adaptive value of the genotype is manifested in the fitness of the phenotypes which it engenders in its carriers when the latter develop in certain environments. It is not the genotype but a living individual who exhibits a certain succession of phenotypes at different stages of its life, and who survives or dies and succeeds or fails to reproduce. And, as pointed out above, the success or failure are decided by the competence of the phenotype not merely in the adult but at all stages of the life cycle. No matter how healthy may be an infant or a larva or a seedling, the Darwinian fitness of the organism is zero if the adult is inviable or sterile. Conversely, high infant mortality is a drawback even if the survivors are vigorous as adults.

ADAPTEDNESS AND THE DEVELOPMENT PATTERN

The phenotype of an individual may be said to follow a certain trajectory in time; this trajectory is determined by the interactions of the genotype with the succession of the environments encountered in the process of living. At any given point on the trajectory the phenotype is decided by its preceding state and by the existing environment. For example, the phenotype of a person at a given moment is the outcome of transformations of his past phenotypes and the base of the future ones. The succession of the changes in a person's phenotype is determined, of course, by the development pattern induced by his genotype and by the successive conditions of the environment.

Let us now consider not the phenotype as a whole but a trait, such as the weight or the state of health or the disposition of a person. Obviously, my weight, health, and disposition are at this moment what they were a moment or several moments ago, except as changed by the ageing process, by diet, weather, occupations, contacts with other persons, etc. I have not inherited any particular "weight," since my weight has varied greatly during the embryonic and post-embryonic life. What I have inherited is a genotype which determines, jointly with the succession of the environments, the trajectory which my weight has followed thus far, and will follow during the remainder of my life.

Sheldon (1954) has attempted to trace the trajectories of weights at different ages and for different body heights for all his somatotypes. These trajectories apply, as acknowledged by Sheldon himself, to development patterns only in environments which are regarded as normal or standard. In other environments the weights may be either higher or lower, and it is gratuitous to regard any weight as inherent or intrinsic for a given somatotype or genotype. All weights which a person may have in any environment, normal or abnormal, salubrious or morbid, are equally inherent and fixed by the norm of reaction of a given genotype to a given succession of environments. This in no way reduces the importance of knowing just what weights individuals of a given somatotype will develop under different circumstances, since different people show different development patterns in environments which are very nearly similar. The same holds for intelligence or for any other trait. There is no such thing as "intrinsic intelligence," unless this is arbitrarily defined as that evolved in a certain environment; neither is there any known upper or lower limits of intelligence, unless certain environments are considered to be the only possible ones. All this does not mean that intelligence is not conditioned genetically; it is, for each set of environments. It would be invaluable to know how persons of different genotypes would develop in different environments, and knowing this should be the aim of experiments with different educational systems.

Although no phenotypic trait is any more "intrinsic" than any other, certain phenotypes exhibit a fitness superior to that of others in the range of the environments in which the species normally lives. The superior developmental patterns and superior phenotypic trajectories are promoted by natural selection, while the inferior ones fail to be perpetuated. Therefore, certain developmental patterns become established in a majority of individuals of every species or population as its adaptive norm. Genotypically conditioned deviations from this norm are hereditary diseases and malformations. Some of them may be "cured," that is, forced back to the normal development trajectory by special environments which are not required by the genotypes which constitute the adaptive norm. Others are incurable, inasmuch as no environments capable of returning them to normal phenotypic trajectories are known.

The fact of crucial importance is that the development patterns which result in adaptively competent phenotypes are homeostatic. They are protected by being buffered against the disruptive effects of environmental changes which are commonly met with in the habitats in which the species normally occurs (Lerner 1954, Dobzhansky and Wallace 1954, Dobzhansky and Levene 1956, Lewontin 1956, and others). Rare or abnormal genotypes, such as homozygotes in a normally outbred species, are often deficient in homeostatic buffering. Nor is homeostasis necessarily shown when normal genotypes are exposed to environments which are unusual for the species. As pointed out especially by Schmalhausen (1949), unusual environments often provoke harmful morphoses instead of adaptive modifications. A non-hereditary disease is, then, a deviant phenotype resulting from the exposure of a normal genotype to an abnormal environment, or a phenotype brought about by senescence which results in a gradual loss of homeostatic adjustments.

A qualification which must be made at this point is that the adaptive norm need not be monolithic; on the contrary, many species and populations are polymorphic, with two or more phenotypic norms which may possess superior fitnesses in different ecological niches. Such polymorphic populations show, then, two or more homeostatically buffered developmental patterns; the development is turned to one of the alternative trajectories by "switch genes" with clear-cut phenotypic effects, or by environmental stimuli (Mather 1953 and others). But whether monomorphic or polymorphic, the developmental patterns which confer high Darwinian fitness upon their possessors are hedged against environmental disturbances which are met in the usual habitats of the species.

CONCLUSIONS

The notorious difficulty of discovering the adaptive significance of traits in which races and species of animals and plants commonly differ is due in part to an incorrect statement of the problem. "Traits" have no adaptive significance in isolation from the whole development pattern of the organism which exhibits them at certain stages of its life cycle. The phenotype of the individual changes relentlessly from conception to maturity and to death; the path which these changes follow is set by the genotype interacting with the succession of the environments to which an individual is exposed. A "trait" may, then, be defined as an aspect of all or of a certain portion of the path or the trajectory of the development. However, it is the trajectory as a whole which confers upon the genotype or the individual its fitness to survive and to reproduce.

When the phenotypic manifestations of two or more genotypes are compared, it may appear that they differ in adaptive values because of some few aspects, or traits, of their development patterns. Yet we must beware of thinking of a development pattern as though it were a sum of independently variable traits. When we wonder why a proclinate oral bristle has not become reclinate, or vice versa, in some species of Drosophila, we implicitly assume that the position of a bristle is decided by a genetic factor or a developmental event which is inserted among other factors and events, but which is not a part of any organized pattern of interrelated processes. Such an assumption is more likely to be wrong than right.

The same assumption is implicit when it is asked why some vestigial organs of the human body have not disappeared completely, as some of them have done in other species, or vice versa. We still possess traces of the muscles that in other mammals move the ears, still harbor the coccyx, an utterly useless relic of the ancestral tail, and some of us are troubled by the vermiform appendix of the coecum, an organ which, for example, the cats have disposed of entirely. Mammalian embryos are full of organs which are formed seemingly only to disappear without ever functioning; the gill bars and the notochord seem to be used only as treasured examples of the biogenetic law and as "proofs" of the occurrence of evolution. However, a complete disappearance of these useless organs, both in the adults and in the embryos, would necessitate radical changes in the basic embryonic processes of organ formation and differentiation. The coccyx is a part of the axial skeleton, and the gill bars arise in the process of formation of the head and the neck. The problem should, therefore, be turned around, and it may be asked what advantages would accrue to the organism from such radical alterations of its development. The development is more than summation of "unit characters" or unit processes. As emphasized especially by Schmalhausen (1949), development is a highly integrated process, and progressive evolution from the lower to higher organisms has on the whole tended to make the integration more and more thorough, and to make the basic developmental processes more and more autonomous from direct stimuli emanating from the environment.

SUMMARY

A trait is an aspect of the whole or of a certain portion of the developmental pattern of the organism. An adaptive trait is, then, an aspect of the developmental pattern which facilitates the survival and/or reproduction of its carrier in a certain succession of environments.

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A COMPARATIVE STUDY OF ANTS IN DESERT REGIONS OF CENTRAL ASIA AND OF SOUTH AMERICA

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INTRODUCTION

The basic postulate of the modern theory of evolution is that the environment furnishes the challenges to which living organisms may respond by adaptive transformations of their structures and functions, these transformations mediated by the processes of mutation, sexual recombination, and natural selection. It follows, then, that similar environmental conditions should furnish similar challenges, and might provoke similar responses. Similar physical environments occur in different parts of the world, often separated by extensive territories in which quite different conditions are prevalent. Thus, the desert belts of the Northern Hemisphere of the Old World (Sahara, Arabia, Iran, Turkestan, Mongolia), and of the New World (western North America) are separated by oceans or by tropical forests and savannas from each other and from the deserts of the Southern Hemisphere in South America, southern Africa, and Australia. It is well known that analogous forms of life, such as, for example, xerophytic plants with fleshy stems and reduced leaves, have evolved in different deserts and often from quite unrelated plant families, such as among Cactaceae in America and Euphorbiaceae in Africa. Nevertheless, it would be an exaggeration to say that similar environments always evoke similar responses in the organisms which inhabit them, and these lacks of parallelisms are perhaps just as interesting for our understanding of the evolutionary mechanisms as are the instances of parallel developments.

The present article compares the ants which inhabit the desert regions of Asia, particularly Turkestan, and of South America, particularly Argentine. It is based on personal observations of the author (Turkestan 1922/1934, Argentine and Bolivia 1947–1955). The desert regions compared are separated widely by seas and by continents with more humid climates. The desert regions merge into adjacent more humid zones; the Argentine desert is adjacent on the north to the tropical part of South America, and on the west to the humid southern Chile; the deserts of Turkestan are bounded by the mountains to the east and to the south, and by the steppes of southern Siberia to the north. The existence of more or less wide intermediate zones facilitates the evolutionary connections between the deserts and the adjacent non-desert zones.

TURKESTAN AND ARGENTINE DESERTS COMPARED

The outstanding similarities between the zones compared are due, of course, to their aridity. Otherwise they differ in a number of respects. (A)

Turkestan is an eastern extension of the Mediterranean region lying approximately between the latitudes 37° and 40° N. The principal constituents of the ant fauna are more or less the same throughout, from Turkestan to northern Africa. The ant fauna of Tashkent resembles that of Iraq (Weber 1952). The desert region of Argentina is elongated meridionally, from the Strait of Magellan to the Tropic of Capricorn, and farther northwards to the high plateau of Bolivia and the Pacific Coasts of Chile and Peru. (B) The deserts of Turkestan are chiefly low plains, and at elevations higher than 500m in the adjacent mountains there appear mesophilic elements. Argentine deserts start at the sea level in Patagonia, but reach elevations of 5000m and higher in the North. The fauna of the Altiplano of Bolivia resembles that of Mendoza and of Patagonia. Some ants (*Camponotus Bruchi* Forel) occur as high as 4500m a.s.l. By contrast, the ant fauna of high Tibet is very different from that of Turkestan; the former has an only slight admixture of mediterranean elements, and is essentially that of the boreal type (the genera Myrmica, Formica, etc). (C) The deserts of Turkestan are isolated from the humid tropical zones, while those of Argentine merge into more humid regions, to the tropical forest inclusive. (D) The deserts of Turkestan are humid and cold in winter (the absolute minimum temperature in Tashkent is −29.5°C); those of Argentina are dry but less cold in winter, and have rains chiefly during the summers (the absolute minimum for Mendoza, elevation 768m, registered between 1928 and 1937 is −8.0°C). The overwintering conditions for the ants are evidently different.

Because of the above differences, one may expect that the ant faunas of the deserts in question would be derived from philogenetically different sources, but should show some parallelisms because of adaptation to similar habitats.

THE CLASSIFICATION OF ANTS

As a basis for the discussion to follow, a brief review of the nine subfamilies into which the family of ants (Formicidae) is divided must be given here.

A. *Relatively primitive subfamilies.* Workers and females with a well developed sting. Females more or less resemble workers; the latter are monomorphic. Larvae are active, and are able to feed independently. The colonies are small. Cooperation between the workers from the same colony little developed.

1. *Ponerinae.* Mainly termophilic or mesophilic. Absent in the desert of Central Asia, in Argentina represented by a single species, *Ectatomma quadridens*, relatively little adapted to desert habitats.
2. *Cerapachyinae.* A rather small termophilic or mesophilic group. Not represented in deserts, at least in both areas compared.
3. *Myrmeciinae.* One of the primitive subfamilies, which occurs in a variety of environments only in Australia (bulldog ants, sergeant ants).

TABLE 1

SYSTEMATIC POSITION OF THE ANTS WHICH INHABIT DESERTS OF CENTRAL ASIA AND OF ARGENTINE

Subfamily	Genus	Central Asia	Argentine	Eastern Patagonia
Ponerinae	Ectatomma	–	+	–
Dorylinae	Eciton	–	+	+
Pseudomyrmecinae	Pseudomyrmex	–	+	–
Myrmicinae	Pogonomyrmex	–	+	+
	Elasmopheidole	–	+	+
	Pheidole	+	+	+
	Messor	+	–	–
	Monomorium	+	–	–
	Solenopsis	–	+	+
	Oedaleocerus	–	+	–
	Crematogaster	+	+	–
	Cardiocondyla	+	–	–
	Tetramorium	+	–	–
	Wasmannia	–	+	–
	Atta	–	+	–
	Acromyrmex	–	+	+
	Mycetophylax	–	+	+
Dolichoderinae	Tapinoma	+	–	–
	Araucomyrmex	–	+	+
	Conomyrma	–	+	+
	Dorymyrmex	–	+	+
	Forelius	–	+	+
Formicinae	Brachymyrmex	–	+	+
	Plagiolepis	+	–	–
	Acantholepis	+	–	–
	Proformica	+	–	–
	Cataglyphis	+	–	–
	Formica	+	–	–
	Camponotus	+	+	+

TABLE 2

NUMBERS OF GENERA BELONGING TO VARIOUS SUBFAMILIES IN THE DESERTS OF CENTRAL ASIA AND OF ARGENTINE

Subfamily	Turkestan	Argentine
Ponerinae	–	1
Dorylinae	–	1
Pseudomyrmecinae	–	1
Myrmicinae	6	10
Dolichoderinae	1	4
Formicinae	6	2
Total	13	19

B. *Adult ants morphologically primitive, larvae specialized and are fed by the workers.*

4. *Pseudomyrmicinae.* Mostly wood ants, absent in Central Asia. In Argentine two species are known: *Pseudomyrmex gracilis* (living in wood) and *Ps. denticollis* (in the soil). Both species related to forms living in the tropical zone to the north.

C. *Phylogenetically more advanced groups.*

5. *Leptanillinae.* A small group related to the following.

6. *Dorylinae.* Nomadic predators forming very large colonies, but usually having no permanent nests (there are, however, some exceptions). Widespread in the tropics both of the Old and of the New World. Not represented in the deserts of Central Asia; in the deserts of India, western Asia, and Africa represented by the genus Dorylus; in the Argentine deserts represented by the genus Eciton, the species of which have close relatives in the tropics of South America.

D. *Most highly evolved groups.* The cooperation between the workers of a colony is highly developed. Showing a tendency towards a plasticity of behavior at different ages, contrasting with the gregariousness of the preceding subfamily. Larvae are fed by the workers. Often showing complex biosocial relationships between different species and genera (slave makers, social parasites).

7. *Myrmicinae.* Workers and females with a sting which may be well developed or more or less strongly reduced (Attini). This subfamily occurs in humid as well as dry, hot as well as cold countries; abundantly represented in South America.

8. *Dolichoderinae.* The sting is absent in both females and workers. This subfamily seems to have been dominant during Tertiary times (Baltic amber, Florissant), now abundant only in Southern Hemisphere and very common in deserts of Argentina.

9. *Formicinae.* Females and workers without a sting. The dominant subfamily at present, most developed in the Northern Hemisphere and in the tropics of the Old World, less so in South America.

SPECIALIZED ECOLOGICAL TYPES OF DESERT ANTS

1. *Hunting ants.* Ants which catch their prey running on the surface of the soil. They develop considerable speeds in sunny weather and at high temperatures. The hunters are working singly, not forming groups of columns of individuals. This ecological type is characterized by an elongation of the thorax and of the legs as well as by a well developed psammophore. Moreover, they frequently have adaptations which permit them to avoid excessive heating of the body tissues even with intense activity during the noon hours. Among these adaptations the most conspicuous ones are pale body colors, dense silvery pilosity, and, in some species of Cataglyphis, reducing the surface of the integument facing the sun by holding the abdomen vertically upwards. It may be noted that these adaptations tend to be alter-

natives: the forms which have the habit of holding the abdomen vertically usually lack the pale colors and the silvery pilosity.

2. *Granivorous ants.* This ecological type, at its most highly specialized level, is characterized by the following traits. (A) The workers have more or less massive bodies with relatively short but powerful legs; the locomotion tends to be slow. (B) The chief or sole source of food are seeds. (D) The seeds are transported to the nest over trails which radiate from the nest's entrance. (E) Inside the nest the seeds are cleaned of glumes and other protective covers, and the covers are removed from the nest forming accumulations near the entrance. (F) The seeds are stored in subterranean chambers, and the ants prevent their germination; the occasional seeds which do germinate are thrown out of the nest. (G) The ants work in organized groups, forming columns of workers who transport the seeds to the nest. (H) These columns may be very large, numbering thousands of individuals. In less specialized representatives of this ecological type some of these traits may be absent.

3. *Ants which cultivate fungi.* The most highly specialized forms of this type (the genus Atta) possess the following traits. (A) Colonies are large, consisting of thousands of individuals. (B) Nests are subterranean, with numerous fungus "gardens," each in a separate chamber. (C) The nests have a system of subterranean galleries serving different functions, such as transportation of the leaves, extraction of the used-up mycelium, ventilation, etc. (D) Subterranean chambers for refuse (used-up mycelium, remains of the dead ants, etc). (E) The nests have many openings, from which radiate trails over which move the workers procuring leaves on which the fungi are to be grown. (F) A division of labor among morphologically differentiated workers; small workers cultivate the mycelium and take care of the larvae, larger ones collect leaves and transport them to the nest, the largest of all are "soldiers" which defend the nest from intruders. (G) The workers work in groups the members of which cooperate with one another.

In the more primitive representatives of the same ecological type the workers are uniform, the colonies are small, and use feces of caterpillars and other insects as the substrate for the cultivation of fungi.

HUNTING ANTS IN THE DESERT OF CENTRAL ASIA AND OF ARGENTINA

As an ecological type, a hunting ant seems to be a simpler adaptive system than a granivorous, and especially a fungus-growing form. Hunting ants occur even among the primitive subfamily Ponerinae, although they do not reach high levels of specialization. *Ectatomma quadridens* is a hunter belonging to the above subfamily, which occurs in the Argentine desert. This species does not, however, penetrate the most arid parts of the desert, being confined to the marginal northeastern zone. It interrupts its activity during the hottest hours of the day, and lacks the adaptations which prevent the overheating of the body. Species related to it are mesophilic.

More highly specialized hunting ants have evolved both in the deserts of Argentine and in those of Central Asia. But while in the former region they belong to the subfamily Dolichoderinae, in the latter region they have developed in the subfamily Formicinae. This is a splendid example of a convergent development of similar ecological types in quite different phyletic lines.

In Central Asia the hunting ants belong to a single genus, Cataglyphis, which does not occur in the New World at all. This genus is a member of a group of rather closely related forms the relationships between which may be represented as in figure 1.

This entire group of forms belongs to the Old World, has some representatives in the temperate and cold areas of North America, and is quite foreign to South America. The morphological differences between Proformica and the less highly specialized Cataglyphis amount chiefly to a change in the body proportions towards elongation of the body and of the extremities (in Cataglyphis). *Cataglyphis cursor* lacks the adaptations characteristic of the desert hunting ants: its coloration is dark, the abdomen is held horizontally, no silvery pilosity. It lives only at the periphery of the arid zone and in mountains. *C. pallidus* already has a pale color, matching that of sand, and it lives only in sandy deserts. Among the more specialized Cataglyphis, *C. albicans* and *C. bicolor* hold the abdomen vertically upwards. Both species are common in the deserts of Central Asia. *C. bombycinus* shows a dense silvery pilosity, and the rapidity of its movements reaches the upper limit among ants. It lives in the deserts of Africa.

The hunting ants of Argentine deserts belong to the genus Dorymyrmex. The phylogenetic relationships of this genus can be represented as follows:

Iridomyrmex
↓
Araucomyrmex
↓
Dorymyrmex

Iridomyrmex is a pantropical genus, well represented in the Baltic amber, and at present most highly differentiated in Australia, where some desert-dwelling species are known. It is represented in Argentine by the mesophilic *I. humilis*, which has been introduced and became a pest in Mediterranean countries, in Hawaii, and in other places. Araucomyrmex is a close relative of Iridomyrmex, living in Chile, Patagonia, and in the Andes till about the 30° latitude South. It lives, consequently, only in zones adjacents to the deserts having more or less temperate climates.

Dorymyrmex is a derivative of Araucomyrmex adapted to live in deserts. Its species occur in the Argentine deserts, in the Bolivian and Paraguayan Chaco, and in drier upland valleys of Bolivia, up to 3000m elevation (near Cochabamba). It is not found in Chile. The North American species which are sometimes placed in the genus Dorymyrmex actually belong to a different genus, Conomyrma.

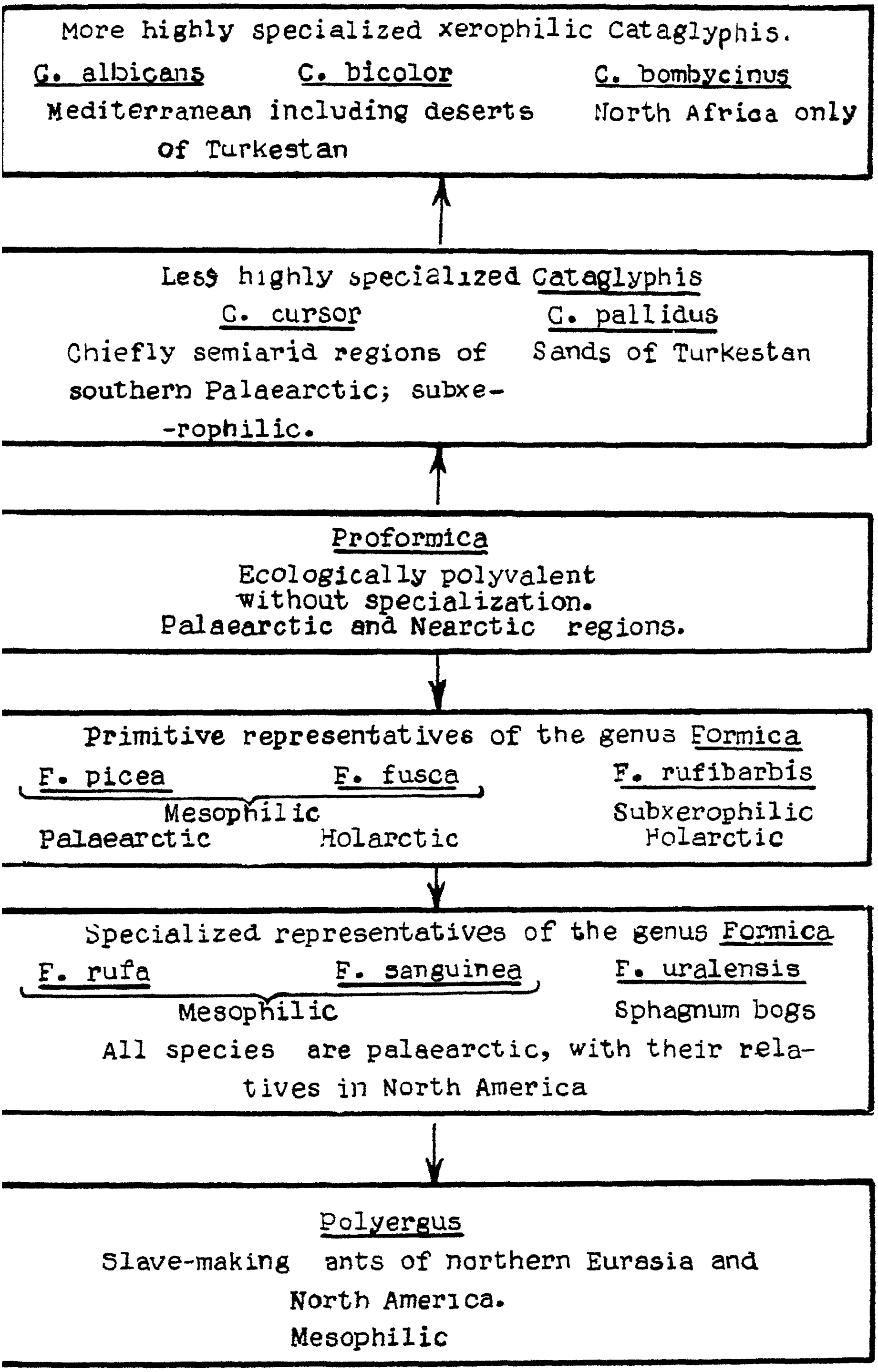
More highly specialized xerophilic Cataglyphis.
C. albicans
C. bicolor
C. bombycinus
Mediterranean including deserts of Turkestan
North Africa only
Less highly specialized Cataglyphis
C. cursor
C. pallidus
Chiefly semiarid regions of southern Palaearctic; subxerophilic.
Sands of Turkestan
Proformica
Ecologically polyvalent without specialization.
Palaearctic and Nearctic regions.
Primitive representatives of the genus Formica
F. picea
F. fusca
F. rufibarbis
Mesophilic
Palaearctic
Holarctic
Subxerophilic
Holarctic
Specialized representatives of the genus Formica
F. rufa
F. sanguinea
F. uralensis
Mesophilic
Sphagnum bogs
All species are palaearctic, with their relatives in North America
Polyergus
Slave-making ants of northern Eurasia and North America.
Mesophilic

FIGURE 1.

The point which is most important for our purposes is that in the evolution of Dorymyrmex one finds the same adaptive trends which we have seen in the systematically as well as geographically remote Cataglyphis. These tendencies are: elongation of the body and the extremities, pale coloration, and reduction of the body surface exposed to the sun. Interestingly enough, the development of these adaptive traits has failed to reach in any species of Dorymyrmex the high levels which it has reached in Cataglyphis. Roughly speaking, the most highly advanced Dorymyrmex have not exceeded the level of desert adaptation found in the relatively unspecialized *C. pallidus* (see above). Species of Dorymyrmex are not very rapid runners, and their activity usually becomes somewhat depressed during the hottest hours of the day.

GRANIVOROUS ANTS IN THE DESERTS OF CENTRAL ASIA AND OF ARGENTINA

Granivorous ants have developed in the arid zones all over the world, but, interestingly enough, they have developed from different phylogenetic sources in different regions, although they all belong to the same subfamily, Myrmicinae. In the deserts of Central Asia this ecological type is represented by the genus Messor, the species of which possess in highly developed form all the specialized traits of the granivorous type enumerated above. *M. barbatus* is the dominant ant species not only in Turkestan but also in deserts of western Asia and of northern Africa; this is the ant which attracted the attention of the ancients and deserved a mention in the writings of King Solomon.

In the Argentine deserts the situation is quite different. At least four different genera have "attempted" to solve the "problem" of evolving a granivorous ecological type, yet not one of them succeeded in reaching the level of specialization found in the genus Messor of the Mediterranean region. The nearest approach to Messor is found in the genus Pogonomyrmex, which contains some fairly large, massive bodied ants with short but strong extremities. The workers of Pogonomyrmex are monomorphic or with only slight polymorphism (*P. coarctatus*). The morphological parallelism between Messor and Pogonomyrmex is emphasized by the presence of the so-called psammophore. The Argentine species do not build trails for the transport of seeds, and do not make large stores of seeds in the subterranean chambers of their nests.

The genus Pogonomyrmex is exclusively American, having two disjunct distribution areas, one in the Argentine and the other in the United States, in both countries living chiefly in arid territories (Kusnezov 1951). This genus is known in fossil condition (Florissant beds, Carpenter 1930), and its high taxonomic differentiation in the Argentine suggests that it is geologically ancient. Its failure to evolve highly developed granivorous specializations cannot, therefore, be explained either by its youth or by a young geological age of the deserts of Argentine (Kusnezov 1951).

The phyletic roots of the genus Pogonomyrmex are to be looked for apparently in the American tropics, where are found living at present the mesophilic relatives Hylomyrma, Forelomyrmex, etc. This inference is supported by the distribution of the genus Ephebomyrmex, which represents a branch of a phyletic trunk common with Pogonomyrmex. Ephebomyrmex has several species in tropical and subtropical parts of South America, Central America, and in Southern United States; species endemic to the Antilles; and a group of 3 peculiar species in the humid western Patagonia and southern Chile. The distribution area of this genus is, then, discontinuous; its species do not occur in arid zones, or if they do are rare there. The "conquest of the desert" by the originally mesophilic forms seems to be clear enough, though the details of this process need more study.

The other three genera which evolved granivorous forms in the Argentine are Elasmopheidole, Pheidole, and Solenopsis. They have small workers and large soldiers with particularly large heads. In every case, the desert granivorous species are clearly related to unspecialized mesophilic ones. Another fact worthy of note is that, in Elasmopheidole and in Pheidole, the degree of the development of the granivorous specialization is positively correlated with a progressive increase in the sizes of the workers and soldiers. Thus, *Elasmopheidole aberrans* and *Pheidole spininodis* live in the most arid regions, sometimes are the dominant ant species in such regions, and are the largest representatives of the respective groups. The increase in size may conceivably be related to the greater complexity and the greater depth of the subterranean nests which are needed in the deserts.

FUNGUS-GROWING ANTS ARE CONFINED TO THE NEW WORLD

This ecological type of ants occurs exclusively in the New World, and is lacking in the Old. The fungus-growers belong to the tribe Attini of the subfamily Myrmicinae (see above). The lack of Attini in the Old World can hardly be accounted for by supposing that they have become extinct there. This tribe is unknown in fossilized condition; moreover in the New World, Attini live in all sorts of geographic environments, and some of its species are among the ecological dominants of the neotropic fauna.

In the Argentine deserts the highly specialized fungus growers are represented by two species of Acromyrmex (*A. lobicornis* and *A. striatus*), and one species of Atta, the precise taxonomic status of which is still unclear. All three species are very common. Nevertheless, they have close mesophilic relatives in the tropical zone. There is no doubt that the "conquest of the desert" has been accomplished by originally mesophilic forms.

Some less highly specialized fungus growers have also succeeded in occupying desert habitats. In the provinces of Mendoza and La Rioja, as well as in other parts of the arid zone, there are commonly found species of the genus Mycetophylax. The nests of these ants are small, with usually a single chamber containing a fungus "garden," but this chamber is located at a considerable depth under the surface of the soil (up to 1½ m and more). The ants are active not only in summer but also in very dry winter. Mycetophylax is, however, the only one among the primitive genera of Attini which succeeded in populating the arid zone, in which it is even more abundant than in the more humid regions. One may conjecture that Mycetophylax is a more ancient migrant from the mesophilic tropical zone into the arid regions than are the more specialized fungus growers. In this connection, it is interesting that fungus-growing ants are absent in Chile. This may indicate that this ecological type is phylogenetically young, and that its absence in Chile may be accounted for by the isolation due to the uplift of the hight range of the Andes. In Bolivia, *Acromyrmex seriatus*, a representative of Attini, lives however at elevations of 3500m and higher. Its existence there may be favored by the intense insulation of the soil during the winter months, which permits uninterrupted growth of the fungus gardens.

It is, then, clear that: (A) Fungus-growing ants represent a highly specialized and statistically improbable form of life. (B) This form of life has been highly successful in the New World, under mesophilic as well as under desert conditions. (C) This form of life has evolved only in the New World, and has no parallels at all in the Old World. Identical, or at any rate similar, challenges have evoked an evolutionary response in this case only in American ants, and no response in the Old World. Clearly, then, an environmental challenge by itself does not guarantee the occurence of uniform response in different places and at different times.

OTHER DIFFERENCES BETWEEN THE ANT FAUNAS OF CENTRAL ASIA AND OF ARGENTINA

Arboricolous ants are absent in Central Asia, and common in the Argentine. An example is *Pseudomyrmex gracilis*, which lives in the tropical South America as well as in the Argentine deserts. Its distribution extends southwards to the province La Rioja, but it is absent in Chile. The arboricolous habit places the ant in a microenvironment which is suitable for essentially mesophilic forms. Arboricolous ants are very common in western Chaco, despite its long and very dry winters.

Deserts of Central Asia lack gregarious nomadic ants of the subfamily Dorylinae, which are not uncommon in the subtropical deserts of northern Argentine and which go southwards to the provinces of Mendoza and Rio Negro.

These differences between the Asiatic and the South-American deserts may be related to the fact that the arid zone of the Argentine merges gradually into the humid tropical zone farther northwards. This facilitates the passage and adaptation of mesophilic forms to the arid zone. Central Asia is, on the contrary, isolated from tropical countries by high mountains. It is, nevertheless, impossible to account for all the differences between the ant faunas of the Asiatic anc South-American deserts on this basis.

One of the important constituents of the Mesophilic fauna of western Patagonia and of southern Chile is the primitive subgenus Notomyrmex of the genus Monomorium. Now, Monomorium has a wide distribution, extending in the Southern Hemisphere to Australia and to New Zealand. It is evidently a very ancient group which has lived for a long time in the southern part of South America. And yet, Monomorium has evolved desert-dwelling forms in Australia, Africa, the Mediterranean Region, Central Asia, but not in South America.

Thus, neither environmental challenges, nor the availability of similar ancestral forms, nor both, are sufficient to guarantee the appearance of similar ecological types adapted to living in deserts. We are forced to visualize evolution as a natural creative process, in which the potentialities of organic forms may or may not be realized through the mechanism of natural selection. Ecologically parallel forms of life may arise from phylogenetically different and sometimes not even closely related sources. A conception of the creative nature of evolution similar to that presented in this paper on the basis of studies of systematics and ecology of ants has recently been developed on a genetic basis by Dobzhansky (1954, 1955).

SUMMARY

1. The ant faunas of the deserts of Central Asia and of Argentina are phylogenetically different in origins, and are related to the faunas of the neighboring non-desert regions.
2. The desert faunas show remarkable parallelisms, due to the presence of similar ecologically specialized types, such as hunting ants and granivorous ants. At the same time, other ecological types are conspicuously absent in one or the other fauna. Thus, fungus-growing ants are absent in the Old World, while the hunting and the granivorous ants have reached higher levels of specialization in the Old than in the New World.
3. These differences and lacks of parallelisms cannot be accounted for either by different geological ages of the deserts compared, or by the presence or absence of suitable ancestral types of ants which could produce forms adapted to desert living. Thus, the genus Monomorium has given rise to desert dwellers in the Old World, but not in deserts of South America.
4. Similar environmental challenges do not, therefore, produce necessarily similar evolutionary responses of the living matter. Evolution is a natural creative process. Phyletically different organic materials may, because of the action of natural selection, yield parallel ecological types. These ecological types become then components of functional systems, which are the faunal complexes of deserts and of other types of habitats in different parts of the world.

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AXIAL ROTATION AND THE ORIGIN OF VERTEBRATES

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The origin of the vertebrates from their hypothetical invertebrate ancestors has never been fully explained. The many theories that have been put forward betoken their inadequacy by their very multitude. Almost every phylum of the invertebrate stock has in its turn been laid under charge as the common ancestor. The present trend of biological opinion turns on embryological evidence, in which, in the opinion of some, undue attention is paid to the minutiae of extremely simple forms. It is not every biologist who agrees with the weighty conclusions that rely on such microscopic detail for their principal if not only evidence. The major features of vertebrate morphology are left untouched by these embryological theories. Chief of these, and probably the most outstanding in relative importance, is the overall pattern of the nervous system.

There is a complete unconformity between the structure of the nervous system in the higher invertebrates and the lowest vertebrate, the lamprey. The invertebrate nerve-cords are ventral, below the gut, but in the vertebrates the gut or venter is below the nerve-cord. Modern biology has rejected the simplest and most attractive interpretation of this anomaly, which is that vertebrates are merely invertebrates walking on their backs (St. Hilaire, 1822), thus bringing the ventrally placed nerve-cord of the worm and the lobster into the dorsal position it occupies in fishes and man. Dohrn's, (1875) "turn over" theory, however, is far from dead, and there are reasons for believing that Axial Rotation may account for much that is otherwise hard to explain; such for instance as the dorsal pituitary of insects, the rivalry between epithalamus and hypothalamus for the leadership of the cross-brain, and above all the presence and structure of the optic chiasma and the decussating systems of contralateral control to which it has given rise.

THE OPTIC CHIASMA

In the invertebrates, the eye or eyes on the one side of the head are in touch with the cerebral ganglion of the same side: there is no crossing over, as with the vertebrates. The cuttlefish, for instance, has an eye so well patterned that it lacks practically nothing, as regards its optical and physiological essentials, of the structure of the vertebrate eye; only the brain behind it is wanting. There is merely a pea-size optic ganglion attached to each eye and connected by its optic stalk with the cerebral ganglion of its own side. This is the highest development of the invertebrate eye.

In vertebrates, however, the eye on the right-hand side of the head (or that part of it, in mammals, which views the right half of the field) is joined to the left-hand side of the brain. This cross-over or decussation of the optic nerves takes place in the midline chiasma. This discontinuity between uncrossed and crossed control, in the transition from invertebrate to vertebrate, is thus as remarkable as that between ventral and dorsal nerve-cords. It is a matter of some concern, therefore, to seek out the evidence afforded by intermediate stages.

The lancelet, having no head and no cranial eyes, has no chiasma. From its invertebrate forebears it has inherited the mathematical regularity of its longitudinal sigmentation. The lop-sided symmetry of some of its near relatives, however, suggests that these transitional forms have not yet fully adapted themselves to the results of axial rotation.

In fishes, the chiasma is of simple type. The two optic nerves cross over without any intermingling or true decussation of their fibres, like the blades of a pair of scissors. Like scissors, therefore, there are two cheiralities or handednesses, enantiomorphic forms which cannot commute except in a mirror. The two forms (right nerve on top, and left nerve on top) occur in almost exactly equal proportions. Parker examined a hundred specimens each of ten common species of symmetrical fish. "In six of the ten fishes examined the left nerve was dorsal about as frequently as the right, the greatest difference being never more than ten per cent. In the remaining four this difference does not exceed in any instance twenty per cent.... In a total of one thousand the right nerve was dorsal in 514 times, the left 486" (Parker 1903). There was no correlation between the dimorphism of the optic chiasma and the other great dimorphism, that of male and female.

This crossing over of the optic nerves could be accounted for by the two eyes maintaining the same position relative to the outside world as they possess among the invertebrates, while the central nervous axis rotated half a turn along with the rest of the body. This rotation presumably took place indiscriminately to right or left in equal proportion, thus explaining the 50-50 ratio discovered in fishes. The cheirality is probably inherited genetically, as it is known to be among many phyla of invertebrates.

The flat-fishes such as the soles and flounders, for reasons unknown, seem to have rebelled against the ancestral pattern of upside-down living, and have attempted to revert to the status antiquior. A flounder starts life as a symmetrical embryo little different in structure from that of a normally shaped fish. With almost catastrophic suddenness, however, it axially rotates through a right-angle and comes to swim on its side. The under eye, with its visual field cut off, rapidly travels round or through the top of the skull to join its luckier fellow on the upper side, trailing after it the optic nerve (Williams, 1902).

If the axial turn from upright to flat is in the same direction as the previous rotation (of two right angles) that brought the optic chiasma into being, the two optic nerves will be given an extra twist and will be interlaced by a third and additional right-angle. If, on the other hand, the ontogenetic rotation is in the reverse sense to the phylogenetic rotation, the optic chiasma will be unwrapped to the extent of one right-angle. In the American flounder, the latter condition is invariably the case, and no specimens are recorded in which the chiasma is further complicated by the rotation. It might be thought that this is due to a process of selective mortality which eliminates all the embryos that have rotated in the "wrong" direction; this, however, is not the case, for the cheirality of the flounder is predetermined in its unhatched embryo, all of whom are of the same monomorphic pattern (Parker, 1903).

There is some evidence that this crossing over of the optic nerve is biologically a disadvantage. Certain species of flat-fish exhibit both dextral and sinistral forms, that is they rotate so that the right or the left side comes to lie uppermost. Rarely, a left-handed halibut is found; the Californian halibut and the stellate flounder are almost as frequent one way as the other. The number of sinistral individuals of the European flounder varies from 5 to 36 per cent in different localities and age groups. In the embryos of each of these species, however, the crossing of the optic nerve is uniformly monomorphic, either dextral or sinistral according to its kind. Many specimens reach maturity, therefore, with an over-twisted chiasma instead of the untwisted type. Population-counts of the European flounder at Plymouth indicate that the handicapped fish die at an earlier age than the others (Duncker, 1900). It is reasonable to argue from this that if a three-right-angled chiasma carries a higher mortality than a one-right-angled chiasma, the fact of twisting is itself a mechanical disadvantage. The normal vertebrate chiasma perhaps may carry somewhat of this handicap by virtue of its two-right-angles of twist. It is presumably to offset this structural weakness in the vertebrates that the complicated systems of sensory and motor decussations have evolved, leading to the contralateral control of each side of the body by the opposite side of the brain. This has sometimes led to anomalous results: a trout blinded in the right eye will darken on the left side of the body, which obviously can serve no useful purpose in protective adaptation (Frisch 1911).

THE PINEAL BODY

The mystery which has surrounded the pineal gland since the days of Descartes, who lodged therein the theological soul, has if anything been enhanced by the efforts of biologists (among them Gaskell in chief, 1908) who would discern in this rudimentary organ a leading clue to the gap between the invertebrate and vertebrate kingdoms. There are good reasons for considering the pineal-epithalamic complex on the roof of the third ventricle to be the enantiologue of the pituitary-hypothalamic complex on its floor. The pineal recess of the third ventricle corresponds to the hollow of the infundibulum; the glandular pineal body or epi-physis represents the neuro-pituitary or hypo-physis and, like that gland, has some part in the control of sexual development; the habenular ganglion corresponds (at least in mammals) to the hypothalamus.

The surviving pineal eye (of lampreys and reptiles) is as distinct from the solid pineal gland (of birds and mammals) as the paired lateral eyes are distinct from the pituitary gland. No animal possesses both structures (glandular and visual) in its epithalamus; there has been a tendency, therefore, to regard all unpaired, midline structures in this position as homologues, and to say that the pineal gland of mammals is the representative or rudiment of the pineal eye of lower forms.

The life-story of the lamprey recapitulates in five years the early history of the vertebrate kingdom. The lamprey has in the roof of its head two pineal eyes, right and left, one of which (the left) has degenerated and rotated to the midline anteriorly, the other (the right) is functional and has rotated to the midline posteriorly. Each eye has or had a pineal optic nerve which passes into the habenular ganglion of its own side, and thence to the optical centres in the roof of the mid-brain. The two nerves, starting above from the eyes fore and aft, rotate as they descend and come to lie side by side: rotate, that is, to form a semi-chiasma of one right-angle (Gladstone, 1940). This is the condition reached (from the opposite direction) in the dextral flounder which untwists its optic chiasma.

The pineal eyes of the lamprey are a direct inheritance from its unknown invertebrate ancestors. This is proved by two salient facts. Invertebrate eyes, starting as an invagination of the outer skin, have the pigment-cells superficial, the nerve-cells deep—the so-called erect type of retina. Vertebrate eyes likewise start as a pigmentation and dimpling of the skin, close to the midline of the back; at a very early age (in the human, within three weeks of conception) the whole midline strip of skin is itself invaginated to form the neural tube, and the optic dimple or cup, increasing its depth, is now an evagination from the neural tube rather than an invagination from the skin. "The human optic pits are two lateral out-pouchings of the anterior end of the neural tube. Since the cephalic extremity of the tube is still open at the time of their formation they actually form ectodermal depressions on the surface of the body. When the roof of the neural tube is ultimately closed, these evaginations enlarge and...form two lateral projections on the ventro-lateral aspect of the brain (4 mm stage)" (Duke-Elder, 1932). Thus it grows toward the surface, nerve-cells or deep side first, and the retina is said to be inverted. The pineal eyes of the lamprey are erect, that is of invertebrate type, with the pigment cells superficial, the nerve cells deep.

Moreover, each pineal eye is connected to the habenular ganglion of its own side, as in the cuttlefish; there is no decussation and no contralateral control. The right habenular ganglion, of the lamprey corresponding to the surviving and functional eye, is much larger than the left. In reptiles, it is the left eye which functions, the right which degenerates; the left habenular ganglion accordingly is the larger, proving that control is predominantly ipselateral.

In its early larval stage, the lamprey has only pineal eyes, its epithalamus is in control of its daily cycle of color-change, and its hypothalamus and hypophysis are immature. Later it grows into the vertebral pattern, its lateral eyes develop and its pineal eyes undergo degeneration. Why this degeneration should occur in the lamprey and in all vertebrates is not known. A zenithal eye is not of great use; the pineal complex is buried by the overgrowth of the cerebral cortex; the skull-bones enlarge and thicken to give support to the temporal muscles that move the biting jaws: all these have been suggested and all may be true. It is more than likely that the pineal eyes, finding their outlook reversed after axial rotation, and the visual reflexes they control taking place all in the wrong direction, attempted a kind of neurobiotaxis to correct the error and to regain their wonted fields of view. In this the two eyes were successful only to the extent of one quarter of a turn; their half-completed journey, which brought them both to the midline-position, was never finished owing to their supercession by the lateral eyes on the other side of the brain. Whatever their fate, there is no doubt that the pineal and invertebrate eyes of the lamprey are embryologically, as they are racially, the earlier of the two systems.

The paired eyes, and their optic nerves which decussate at the chiasma, have been explained as having arisen in the earliest vertebrates as a consequence of axial rotation. It is remarkable, therefore, that the neural centres of the ventrally placed optic nerves are the same as those of the dorsally placed pineal nerves of the roof of the mid-brain: the optic fibres have to encircle almost the whole girth of the brain-stem, after leaving the chiasma, to arrive at their destination. A few of the visual afferents, however, succeed in making contact with the hypothalamus, just as the bulk of the pineal nerves enter the habenular ganglion, and this is a further reason for regarding the one system as the enantiologue of the other.

Both epithalamus and hypothalamus have intimate connections with the olfactory centres: the dorsal nuclei through the striae medullares, the ventral through the medial forebrain bundle. The division of the rhinencephalon itself into a degenerate dorsal half and a functioning ventral half may be related to the division of labor in the cross-brain resulting from axial rotation and the refashioning of the visual system, leaving the ventral hypothalamus functioning and the dorsal epithalamus degenerate.

CONTROL OF RHYTHM

The pineal gland of the epithalamus is the enantiologue of the pituitary gland of the hypothalamus. In the larval lamprey, the pineal complex controls the rhythm of the daily color-change from pale-by-night to dark-by-day; when it is removed, the animal remains dark. Not only does the pineal control the rhythmic response to the nyctohemeral rhythm, but it has also internalized this rhythm: when the animals are kept in total darkness "the diurnal changes usually persist, though diminished in extent" (Young, 1935). Removal of the pineal complex largely abolished this internalized rhythm.

In thus mediating the rhythmic response to day and night, and in internalizing the rhythm, the pineal complex of the lamprey shows its affinities both with the pituitary gland of the higher vertebrates and with the corresponding dorsal structure of insects. The paracardiac body of insects is a small knot of neuro-secretory tissue containing three types of cells: chromophil, chromophobe and interstitial. The chromophil cells are probably the source of a colloid substance resembling that in the mammalian hypothalamus; they provide the hormone responsible for color-change in certain insects. Closely associated with the paracardiac body is the corpus allatum which produces a "juvenile hormone," a yolk-forming hormone, and a hormone which has a general effect on metabolism, reminiscent of the polyvalance found in pituitary secretions. The greater part of the complex lies dorsally as regards the pharynx and the ventral nerve-cord.

Certain insects have the power of internalizing a rhythm. The stick-insect Dixippus is dark at night and pale by day, and this daily rhythm has been found to continue for several weeks in complete darkness; it is not inborn, for it fails to appear if the insect is hatched in darkness and kept in the dark. It is an acquired rhythm, and it can be reversed by artificial reversal of the rhythm of illumination; the reversed rhythm will persist in complete darkness just as the natural rhythm. The Chinese fire-fly *Luciola sinensis*, which regularly lights up at seven in the evening, keeps to this habit for several days when kept in the dark. The daily flashings of *Photinus pyralis*, another fire-fly, persist for at least four days. The cricket Gryllus shows a daily rhythm of general activity which will persist at least two weeks in continuous darkness at constant temperature and humidity; like so many others, its rhythm can be reversed by reversed illumination (Wigglesworth, 1950).

It is only an assumption that in insects the paracardiac system which controls the changes of color is the locus for the nyctohemeral rhythm and for the known faculty of internalizing this rhythm. In the crustacea, however, it is known for a fact that the sinus gland (physiologically the analogue of the pituitary) controls the daily rhythm. The fiddler-crab Uca is paler at night than by day, and can maintain this rhythm for as long as 25 days, whether in continued light or total darkness (Abramowitz, 1937); the rhythm is controlled by a glandular structure in the eye-stalk, characterized by blood-sinuses into which it discharges a pigmento-motor chemical resembling mammalian pituitrin. The sinus gland (as it is called) may be regarded as the crustacean equivalent of the pituitary, and there is evidence that it resembles the mammalian gland also in being responsible for calcium regulation and control of growth (Navez, 1934). When the sinus gland is removed (in Uca) the daily rhythm of its color-change is abolished (Abramowitz, 1937).

The reasons are familiar for believing that the human response to natural rhythms is located in the hypothalamus and pituitary. The centres for sleeping and waking are topographically identical (in the rat) with those of the parasympathetic and orthosympathetic in the hypothalamus (Nauta 1946). Many experiments have been made on mammals to test their powers of internalizing the daily rhythm. The longest maintenance of an internal daily rhythm yet recorded was in the white-footed mouse, which kept up a well-defined alternation of activity and rest in an unchanging environment for eighteen months (Johnson, 1939).

The power of internalizing a rhythm is probably as specific a function as any in the nervous system, as characteristic of the tissues concerned as is vision of the eye, and hearing of the ears. In the insect this power resides in the dorsally placed paracardiac centres; in the vertebrate, in the ventrally placed hypothalamo-pituitary complex. Apart from the histological evidence, the imputed homology of these two systems of control is greatly strengthened by the location in both sites of the highly specific power of internalizing an environmental rhythm. The change of position from dorsal to ventral of this neuro-glandular complex is adduced therefore as further evidence for the theory of Axial Rotation.

The lamprey in its five years of adolescence recapitulates the lost story of the earliest vertebrates, in the change-over from dorsal to ventral systems of visual perception. The same animal exemplifies also the change from dorsal to ventral location of the neuro-chemical centres for responding to and internalizing the rhythms of the environment.

SUMMARY

1. Axial Rotation is postulated as the origin of the vertebrates, as this theory alone can account for the reversal from ventral to dorsal of the position of the nervous system.

2. The uncrossed visual control in the higher invertebrates is contrasted with the contralateral control in the vertebrates.

3. The optic chiasma of flat-fish undergoes certain changes during their axial rotation from upright to flat, and these are considered relevant to the theory.

4. The rise and fall of the pineal complex in vertebrate phylogeny is explained by this theory.

5. The enantiology of epithalamus and hypothalamus is related to that of the dorsal (degenerate) and ventral (functioning) halves of the rhinencephalon.

6. The glandular functions of the pineal complex are compared with those of the pituitary.

7. The dorsal pituitary of insects is compared with the ventral pituitary of vertebrates, especially in regard to the power of internalizing an environmental rhythm.

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APPROXIMATE CONFIDENCE INTERVALS FOR SPECIFIC LOCUS MUTATION RATES

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Point mutations, even in irradiated populations, generally occur with extremely small frequencies, and the most difficult obstacle for the average experimenter to overcome is that of obtaining enough data for his results to be meaningful. Because of the sheer number of animals required, very few specific locus mutation rate studies have been conducted and many of these have been restricted in scope. In recent years attention has been focused on the possibility that exposure to radioactive material might represent a hazard to man in terms of its effect on mutation rates. As a result mutation rate studies have been slowly increasing in numbers and in size, and the area of investigation has been extended to the mouse (Russell, 1951). As yet the mouse is the only mammal which has been studied systematically, and it has taken several years to obtain the data which are now available.

Enough data have been accumulated now so that geneticists have begun to make comparisons of radiation-induced mutation rates among species, and because of the urgent need for some estimate of human hazards, no matter how crude, the results are being used to make inferences about human populations. Russell (1951) compared mutation rates in mice and Drosophila on the basis of preliminary results from his own work and the existing literature on Drosophila. No tests of statistical significance were attempted at that time because of the lack of truly comparable data for the two species. Later Ives (1954) discussed the same problem and apparently reached a somewhat different concludion. Recently Russell (1956) has devoted an entire paper to this problem incorporating later data from his own study and new data on Drosophila including experiments (Alexander, 1954) which were specifically designed to provide data for comparison with the mouse. In this case it was possible to make a test of statistical significance. The test confirmed Russell's earlier conclusion that the specific locus mutation rate in the mouse is higher than in Drosophila. There is of course the additional problem of computing confidence limits for these mutation rates and this paper discusses a method for constructing such limits.

The construction of confidence intervals or limits of error for mutation rates obtained in experiments of this kind is complicated by the same factors which complicate the studies themselves. One quantity relevant to the assessment of the genetic effects of radiation is the mutation rate per locus for the population of loci at which mutations can occur in a given species. The number of loci is usually unknown although for most species which have been studied, it is estimated to be in the tens of thousands. Furthermore, the number of known loci at which mutations have been observed is often quite small, and they are usually restricted to such readily observable characteristics as eye color, coat condition, shape of wings, etc. These considerations lead directly to the following two assumptions which would be required for any approach to the confidence interval problem:

(1) The number of loci in the sample is negligibly small compared to the number of loci in the population.

(2) The loci in the sample are chosen at random from the population of loci.

Assumption (1) is certainly satisfied in every case to be considered and means that for practical purposes the size of the locus population can be considered infinite. Assumption (2) is most certainly not satisfied since the loci studied in these experiments are those which have in the past been easily recognized. However, the real intent of assumption (2) is that the method of sampling loci should be such that loci with mutation rates which are either very large or very small, are not preferentially selected. A priori there is little reason to suspect that loci, which when mutated become manifest to the observer, have rates which differ from other loci. In other words there is no reason to suspect a correlation between the magnitude of the mutation rate at any locus and the observability of its phenotype. Thus assumption (2), though not strictly correct, may be accepted and in fact must be accepted if any attempt at constructing intervals is to be made.

If assumption (2) is not satisfied and the result is an upward bias in the point estimate, the effect would be present in any species studied so that species comparisons would presumably not be affected.

In what follows it will be assumed that N males have been exposed to the same dose of radiation and that each survivor is mated to several unirradiated females yielding a total of n offspring from each male. Each offspring is examined for mutations at each of k loci and x_i $(i = 1, \ldots, k)$ mutations are found at the *ith* locus among all offspring examined. It is reasonable to assume that the conditional probability distribution of each x_i is of the Poisson form,

$$g(x|m) = \frac{m^x e^{-m}}{x!} \qquad (x = 0, 1, \ldots),$$

where m is the expected number of mutations at the locus being studied in a sample of Nn offspring. The distribution is conditional in the sense that x will have this distribution only if m is a constant. Actually in the present problem, m is itself a random sample from the population of loci and will have a probability distribution h(m), $(0 < m < \infty)$. Since k is assumed to be negligibly small with respect to the number of loci in the population, h(m) may be assumed to be continuous. Then the unconditional probability distribution of x is

$$f(x) = \int_0 g(x|m)\ h(m)dm.$$

The parameter for which an estimate is desired is the mean number of mutations in the population of loci, i.e.,

$$\lambda = \int_0^\infty m\, h(m) dm .$$

Furthermore the expected value of x is

$$\begin{aligned} E(x) &= \sum_{x=0}^{\infty} x\, f(x) \\ &= \sum_{x=0}^{\infty} x \int_0^\infty g(x|m)\, h(m) dm \\ &= \int_0^\infty h(m) \left[\sum_{x=0}^{\infty} x\, g(x|m) \right] dm \\ &= \int_0^\infty m\, h(m) dm \\ &= \lambda . \end{aligned}$$

Thus the number of mutations observed at any locus is an unbiased estimate of λ, and so is $\bar{x} = \sum_{i=1}^{k} x_i/k$. In other words, the observed mean number of mutations per locus is an unbiased estimate of the population parameter λ, irrespective of the form of the probability distributions, $g(x|m)$ and $h(m)$.

At first glance one might be tempted to construct a confidence interval for λ by assuming that the total number of mutations, $X = \sum_{i=1}^{k} x_i$, has a Poisson distribution. Clearly this procedure would lead to an interval which is too small, since it fails to incorporate the error imposed on the observed data by variations in the mutation rate at different loci. It includes only the Poisson error of sampling at each locus. The conclusion must be that X does not have a Poisson distribution, and any procedure for constructing confidence intervals based on the assumption that it does is incorrect.

In fact X has a distribution which depends on the unknown probability distribution, h(m), and any correct method must be based on some assumption about the form of this distribution. One of the most flexible continuous probability distributions for variables bounded between 0 and ∞ is the gamma distribution,

$$\frac{1}{(\alpha - 1)!\, \beta^{\alpha}}\, m^{\alpha-1}\, e^{-m/\beta} . \tag{1}$$

This is a two-parameter distribution and is flexible in the sense that it can take many different shapes depending on the choice of the parameters α and β (Mood, 1950). With no information, experimental or theoretical, on which to base a decision, it would seem advisable to choose a distribution of this type for h(m) in the hope that a proper choice of α and β would produce a distribution close to the unknown true distribution. Thus if h(m) is taken equal to (1), the distribution of x is given by

$$f(x) = \int_0^\infty \frac{m^x e^{-m}}{x!} \cdot \frac{1}{(\alpha - 1)!\, \beta^\alpha}\, m^{\alpha-1} e^{-m/\beta}\, dm$$

$$= \frac{1}{x!\,(\alpha - 1)!\, \beta^\alpha} \cdot \frac{\Gamma(\alpha + x)}{\left(1 + \frac{1}{\beta}\right)^{\alpha + x}}$$

This distribution is often called the negative binomial distribution and is sometimes referred to as the Pascal distribution. More often it is found written in the form,

$$f(x) = \frac{\Gamma(\alpha + x)}{x!\,\Gamma(\alpha)} \left(\frac{\lambda}{\lambda + \alpha}\right)^x \left(1 + \frac{\lambda}{\alpha}\right)^{-\alpha}$$

where $\lambda = \alpha\beta$. In this form the mean of the distribution is λ and the parameter α is referred to as the exponent of the distribution.

In an effort to determine whether this distribution would adequately describe the results of mutation rate studies of the kind previously described, seven sets of published data were examined with this purpose in mind. Preliminary calculations indicated that the exponent in the distribution should be taken as one. In this case ($\alpha = 1$) the distribution has the form,

$$f(x) = \frac{\lambda^x}{(1 + \lambda)^{x+1}}. \tag{2}$$

The mean is λ and the variance is $\lambda(1 + \lambda)$. Thus from a sample of k loci, $x_1, x_2, \ldots, x_k$, the quantity

$$\chi^2 = \frac{\sum_{i=1}^{k} (x_i - \bar{x})^2}{\bar{x}(1 + \bar{x})},$$

where $\bar{x} = X/k$, has approximately a chi-square distribution with $(k - 1)$ degrees of freedom under the hypothesis that the sample is drawn from a population whose probability distribution is given by (2). This chi-square test was applied to the seven different sets of data and the results are shown in table 1. Excellent agreement with the hypothesis was found in five sets, and in the remaining two sets, the observed numbers of mutations varied less than would be expected on the basis of assuming (2) as the parent population. In practice then if (2) is assumed to be the correct

TABLE 1

CHI-SQUARE TESTS ON SPECIFIC LOCUS MUTATION DATA

Investigator	Russell (1951)		Alexander (1954)		Alexander (1954)		Patterson (1934)		Mickey (1954)		Mickey (1954)		Ives (1954)	
Material	Mouse Spermatogonia		Drosophila Spermatogonia		Drosophila Sperm		Drosophila Sperm		Drosophila Sperm		Drosophila Sperm		Drosophila Sperm	
Radiation	X-ray		X-ray		X-ray		X-ray		X-ray		Neutron		X-ray	
	L*	#†	L	#	L	#	L	#	L	#	L	#	L	#
Number of Mutations	A	0	ru	0	ru	5	ru	16	ru	3	ru	2		
	B	9	h	2	h	6	h	1	h	1	h	2	h	1
	C	3	th	0	th	4	th	3	th	2	th	1	th	2
	D	6	st	0	st	10	st	8	st	3	st	0	st	6
	P	7	p^p	0	p^p	13	cu	3	p^p	2	p^p	3	p^p	5
	S	22	cu	2	cu	8	sr	4	cu	3	cu	3	cu	6
	Se	0	sr	3	sr	12	e^s	13	sr	7	sr	1	sr	13
			e^s	3	e^s	13	ca	22	e^s	11	e^s	3	e^s	6
Mean $\bar{x}$	6.71		1.25		8.88		8.75		4.00		1.88		5.57	
$\sum(x-\bar{x})^2$	343.43		13.50		92.88		395.50		78.00		8.88		89.71	
$\bar{x}(1+\bar{x})$	51.796		2.813		87.641		85.300		20.000		5.391		36.612	
χ^2	6.630		4.799		1.060		4.637		3.900		1.646		2.450	
P‡	0.37		0.68		>0.99		0.70		0.79		0.97		0.87	

*L = locus

†# = No. of mutations at corresponding locus

‡P = probability of a greater value of chi-square

distribution, it appears that confidence intervals computed therefrom will be correct in most cases, and when not correct will be too large. The latter conclusion follows directly from the observation that in two sets of data, the distribution assumed would actually have a larger variance than is indicated. In no case was the variance smaller than expected. This conservative approach is clearly preferable to the aforementioned method based on Poisson variability which uniformly would give confidence intervals which are too narrow.

If the number (x_i) of mutations at the *ith* locus has the distribution given by (2), i.e., a negative binomial distribution with a mean of λ and an exponent of one, then $X = \sum_{i=1}^{k} x_i$ is known to have a negative binomial distribution with a mean $k\lambda$ and an exponent k (Anscombe, 1950). Furthermore, Anscombe (1948) has shown that for this distribution when k is known, the transformation

$$y = \sinh^{-1}\left(\frac{X + 3/8}{k - 3/4}\right)^{\frac{1}{2}} \tag{3}$$

has certain desirable properties. In particular the variable y will be approximately normally distributed when $k > 2$ and $k\lambda$ is large. The variance of y is approximately $1/4(k-1)$. These properties may be used to place confidence limits on the transformed variable, and the limits so obtained may be transformed back to provide limits for $k\lambda$. Specifically if we take

$$\text{Upper limit} = y_U = \sinh^{-1}\left(\frac{X + 3/8}{k - 3/4}\right)^{1/2} + 2\left[\frac{1}{4(k-1)}\right]^{1/2}$$

$$\text{Lower limit} = y_L = \sinh^{-1}\left(\frac{X + 3/8}{k - 3/4}\right)^{1/2} - 2\left[\frac{1}{4(k-1)}\right]^{1/2}$$

then the approximate 95 percent confidence limits for kλ would be given by

$$X_U = (k - 3/4)\sinh^2 y_U - 3/8$$

$$X_L = (k - 3/4)\sinh^2 y_L - 3/8.$$

The method may be illustrated with Russell's (1951) data on the spermatogonia mutation rate in mice as given in table 1. The total number of mutations at all seven loci is 47, so that $X = 47$, $k = 7$. From formula (3),

$$y = \sinh^{-1}\left(\frac{47 + 3/8}{7 - 3/4}\right)^{1/2} = 1.7374,$$

and the standard deviation of y is

$$\left[\frac{1}{4(7-1)}\right]^{1/2} = 0.2041.$$

Thus

$$y_U = 1.7374 + 2(0.2041) = 2.1456$$

$$y_L = 1.7374 - 2(0.2041) = 1.3292,$$

whereupon,

$$X_U = (7 - 3/4)\sinh^2(2.1456) - 3/8 = 110.67$$

$$X_L = (7 - 3/4)\sinh^2(1.3292) - 3/8 = 18.91.$$

These are the approximate 95 percent confidence limits on the total number of mutations. The experiment was performed with 600r of X-radiation and a total of 48,007 spermatogonia were tested. Thus the point estimate of the experimental mutation rate/r/locus is

$$\frac{47}{7 \times 600 \times 48{,}007} = 23.3 \times 10^{-8},$$

(This figure differs slightly from the figure of 22.1×10^{-8} given by Russell (1956) since he is quoting the induced rate whereas the value given here is not corrected for spontaneous mutations. Alexander (1954) found no spontaneous mutations so that the Drosophila rate given below agrees with that quoted by Russell.) and the approximate 95 percent confidence limits are

$$\frac{18.91}{7 \times 600 \times 48{,}007} = 9.4 \times 10^{-8}$$

and

$$\frac{110.67}{7 \times 600 \times 48{,}007} = 54.9 \times 10^{-8}.$$

Identical calculations with the Drosophila spermatogonia data of Alexander (1954), also shown in table 1, yield a point estimate of 1.52×10^{-8} with 95 percent confidence limits of 0.45×10^{-8} and 3.85×10^{-8}.

The primary purpose of this paper is to present a reasonable basis for the computation of confidence limits from specific locus mutation rate studies. The method proposed has been justified in part by reference to known properties of the sampling errors in mutation numbers, and in part by a test of the model with seven independent sets of data. It has been emphasized that the confidence limits obtained are only approximate and Anscombe (1948) has shown that the estimates may be as much as 10 percent too low as a result of the inverse transformation of y_U and y_L. Nevertheless the method is certainly better than one based on Poisson errors alone, and when limits from two different experiments are compared, as for example, the experiments of Russell (1956) and Alexander (1954) already mentioned, the failure of the 95 percent confidence intervals to overlap should leave little doubt about the significance of the higher mutation rate in the mouse. This is particularly true because the assumptions made tend if anything to produce confidence intervals which are wider than the correct ones. It should be noted that the result of this comparison adds further strength to the test of statistical significance made by Russell (1956) and referred to in an earlier paragraph.

A further point to be made is that the appearance of a locus with an apparently high rate is to be expected in any distribution which results from the compounding of Poisson populations with different means, whether or not the resulting distribution is a negative binomial. In other words the observed variability should be greater than one would expect in sampling from a single Poisson population, and the results in table 1 clearly support this observation. Thus any criticism, such as Ives' (1954), of the observed mutation rate in mice which is based on the contention that any specific locus with a high mutation rate is anomalous can hardly be supported. It is worth noting that even the data presented by Ives (1954) contain a locus (stripe) with a relatively high frequency of mutations and thus fall into the expected pattern.

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SUMMARY

A method is proposed for constructing approximate confidence intervals for specific locus mutation rates. The assumptions on which the method is based have been tested by an analysis of published data on mutation rates, and the results of the analysis indicate that the assumptions are conservative. This implies that if the method is in error, it will lead to confidence intervals which are too large rather than too small. The method is applied to data from radiation experiments with Drosophila and mice, and the results

support a conclusion made by Russell (1951, 1956) that the spermatogonia mutation rate in mice is higher than the spermatogonia mutation rate in Drosophila.

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INDUCTION OF MUTATIONS BY FORMALDEHYDE SOLUTIONS IN *DROSOPHILA MELANOGASTER*

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So far the only effective methods of inducing mutations by formaldehyde treatment in Drosophila germ cells are either by mixing the chemical with the food of the larvae, or by injecting the solution in the body of the adult fly (Auerbach, 1952 and 1953, and Sobels 1954). In the feeding method, the effectiveness of the mutagen can only be tested on the larval germ cells, as attempts to feed adults on formaldehyde-treated food proved to be unsuccessfull. Consequently, the pattern of sensitivity of the germ cells to the mutagenic action of formaldehyde has been but partly determined, mainly by Auerbach. However, the introduction of the mutagen through the alimentary canal has its drawbacks. On the one hand, one can never be sure whether the effect produced is directly due to the chemical used without being changed through reactions with other groups. On the other hand, by the application of the feeding method it is not possible to test for the effectiveness of the mutagen or the sensitivity of the germ cells during the pupal life. Such a test is of importance, as it was shown for X-rays that the most sensitive stage of spermatogenesis occurs during late pupal life, (Khishin 1955).

By the second method, that of injecting the solution into the adult fly, it may not be possible to detect a more sensitive stage than the mature spermatozoa by using the usual brood technique. This is so, for reasons which depend almost entirely on the handling of the flies, and on the mating procedure after treatment. Factors such as the number of females per single male, the brood interval, the type of food used, and many others may conceal—as in fact they did in the past—the sensitive stage. All these factors affect the rate of flow of the spermatozoa, so that the sensitive stage may be diluted, to a greater or lesser degree, by stages of lower sensitivity.

For these reasons the present set of experiments was planned. Formaldehyde is being applied in the form of aqueous solutions, in which different pre-imaginal stages of *Drosophila melanogaster* are simply immersed. Different concentrations of the formaldehyde, and varying length of treatments are tested. This method is being tried on two embryonic stages, two larval instars, and on prepupae and pupae at various degrees of development. The aim is to establish the pattern of sensitivity to the formaldehyde by using the induced recessive sex-linked lethal mutations as the measure of sensitivity, and comparing the results obtained in the first broods of males treated at the above mentioned stages.

The present communication gives the result of an experiment in which formaldehyde solution was applied to pupae.

MATERIAL AND METHODS: The stock used was a wild type Or-K stock. The stage treated was that which after X-radiation gave the highest rate of recessive sex-linked lethals. This stage is about 174 hours from the time of egg laying, (see Khishin 1955). The formaldehyde used was a 40 percent commercial product, and dilutions were made with distilled water.

The treatment consisted of simply immersing the pupae in the suitable concentration of formaldehyde for a certain period. Pupae are kept well immersed into the solution by the aid of a piece of cotton wool itself pushed down under the surface. This is necessary as the pupae tend to float on the surface. After treatment the pupae were washed in several changes of tap water, then transferred into empty vials. A piece of filter paper or cotton wool supplies the necessary moisture and prevents drying.

The limits of the concentration of the formaldehyde solution or the duration of treatment will appear elsewhere. The present treatment, however, was for a period of three hours in 10 percent formaldehyde. With this period of treatment, and concentration of formaldehyde, we got about 50 per cent emergence, and almost all the emerging males were completely fertile.

The emerging males were then tested for the recessive sex-linked lethals by the well known Muller-5 technique. Each male was given two virgin Muller-5 females. Only the first brood was obtained, the brood period being three days. The Muller-5 method was also used to test for the spontaneous rate of sex-linked lethals in the stock. Although the spontaneous rate of mutations had been determined often at the Institute of Animal Genetics, Edinburgh, from which the stock was obtained, it nevertheless, was thought better to retest it under its new environment in Egypt.

RESULTS

The results of the experiment are given in the following tables.

TABLE 1

THE SPONTANEOUS RATE OF RECESSIVE SEX-LINKED LETHALS (OR-K).

No. males tested	No. of Chrom.	No. of Lethals	Lethals %
42	660	2	0.30

TABLE 2

THE RATE OF RECESSIVE SEX-LINKED LETHALS INDUCED IN THE GERM CELLS OF DROSOPHILA MALES TREATED AS PUPAE WITH FORMALDEHYDE

No. males tested	No. of Chrom.	No. of Lethals	Lethals %
45	600	8	1.33

CHI-SQUARE TEST

The χ^2 for the result of the spontaneous and the treated series has a value of 4.24. This value indicates a significant difference at the 5 per cent level.

Thus it seems that this method of immersing pupae in formaldehyde solution is effective in producing sex-linked lethal mutations in the male germ cells of *Drosophila melanogaster*. However, the mutation rate is very low, although it is significantly higher than the spontaneous rate as shown by the χ^2 value. The result of the experiment also showed that the spontaneous rate of mutations did not change under the new conditions.

DISCUSSION

There are a few points of importance one has to remember when interpreting results of this kind. First of all, the rate of mutations induced in the germ cells will no doubt depend to a degree on the concentration of the mutagen that reaches the gonads. This in turn will depend on various factors, the most important of which are the length of treatment and the permeability of the puparium and the other internal tissues and cells. The temperature during treatment should also be expected to affect the results, primarily due to its effect on permeability.

Although the above results indicate the possibility of inducing mutations in the germ cells by immersion in formaldehyde solutions, yet to establish a sensitivity pattern by this method one has to overcome a great difficulty. The desire is to compare mutation rates induced in various stages of spermatogenesis after treatment with formaldehyde. But the various stages of germ cell development occur in more than one phase of the animal. For that reason it is necessary to treat eggs, larvae, prepupae and pupae, and also to treat more than one age within every stage. These different stages differ also in the nature of their outer coverings, the chorion of the eggs, the cuticle of the various larval instars, and the puparium of the prepupae and pupae. Consequently it is reasonable to expect differences in permeability through these outer layers. Besides, the internal tissues and/or aggregates of cells and their physicochemical nature also vary from the egg to the larvae and from the larvae to the pupae, and possibly also within the one stage. All this makes it inaccurate to immerse eggs, larvae and pupae in a certain concentration of the mutagen and then consider the rates of mutations induced as a measure of the differential sensitivity of the germ cells. The comparison will not be correct because the amount and concentration of the mutagen reaching the germ cells will not be the same in the three developmental stages, and possibly also within the same stage.

A possible way of overcoming these difficulties is by a good chemical estimation of the formaldehyde in the testes of samples taken from the treated material. If this could be done quantitatively, then it may be possible to interpret the results on the basis of the concentration of the mutagen in the testis. However, the difficulty now will be to find a quick, easy and sensitive chemical method for the estimation of formaldehyde in the testes. So far, we have tried Schiff's reagent and diphenylamine in concentrated sulphuric acid, merely to test for the presence of formaldehyde in the testis of the treated pupae. Both methods gave negative results.

However, some chemical methods are now being tried, and a technique is being developed to ensure better penetration of the mutagen in the hope of obtaining higher rates of mutations.

SUMMARY

It was possible to induce recessive sex-linked lethal mutations in Drosophila male germ cells by simply immersing pupae in aqueous solutions of formaldehyde.

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BOOK REVIEW

MUTUAL AID AND EVOLUTION

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It is good to see Kropotkin's articles on Mutual Aid, first published in *The Nineteenth Century* between 1890 and 1896, and in book form in 1902, brought again to the attention of students of evolution. With all its faults it is a great human document, an expression of the indignation, shared by many and clearly and popularly expressed by Kropotkin, which is aroused when man is viewed as a creature formed by struggle against his fellows, and when his institutions and societies are seen as results primarily of this struggle. It was T. H. Huxley's essay on "The Struggle for Existence in Human Society" published in *The Nineteenth Century* in February 1888 which aroused Kropotkin's indignation and his articles and later book (it is the 1914 edition with its preface which is now reprinted) were direct replies to Huxley's essay.

For comparison with this re-issue I took down my copy of the original edition of 1902 of Mutual Aid which I read as a student over 40 years ago. My notes recalled the impression it had made upon me, for I too was troubled by the antithesis in man's nature, and had been led to Kropotkin by Graham Wallas' *The Great Society* which I read in the same year 1914. To look at those two books now is to see as in a mirror the young man with that time as a background. Then I saw dimly what seems clearer now—that both views, Kropotkin's and Huxley's, were incomplete and distorted because they confused scientific with moral or ethical questions, and tried to justify their views on both from the same evidence. There are many causes of this confusion. "Aid" and "struggle" appear where we use them in a narrow, anthropomorphic sense to be transitive verbs which imply the kind of consciousness (our own) which seems driven to form ethical judgements. It is an all too common practice for those who aim at objective views justified by the facts and the reasoning method of natural science to use such words without precision and to attribute to a natural process such as evolution meanings and motivating forces which are expressions of human (and often humane) aspirations and fears. Surely we should not adduce the behavior of such stereotyped automata as ants or their societies as evidence for either view; nor should we, as Huxley did, preface our arguments "I am as strongly convinced as the most pronounced individualist can be—." Kropotkin's use of the evidence was determined by his philosophical position as an anarchist for whom cooperation was a substitute for the State, while Huxley's thoroughgoing and unabashed materialism led him to admire equally (so he said) the wolf and its victim the deer. But it is clear that his sympathies were with the winner.

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*Mutual Aid, by P. Kropotkin, introduction by Ashley Montagu with an appendix: The struggle for existence in human society, by Thomas H. Huxley. Extending Horizons Press, Boston, Mass., 1955, 362 pp. cloth bound $3.00, paper bound $2.00.

It gives one pause to reflect how one set of facts, observations generally agreed to, like those underlying the theory of natural selection, may lead in different minds to divergent and irreconcilable meanings. Does the reason for this lie in the different extraneous or personal circumstances under which the bent or set of the mind is formed? And how, if this be an ever present danger, is the ultimate disaster to science to be avoided, whereby the discourse is put upon a plane where decision by reference to the factual observations is impossible? One suspects this happens often in the history of science, and that the struggle to limit the meaning of words precisely to the point requiring decision is far from won.

In perspective the final question remains: what have these differing attitudes of Kropotkin and Huxley to do with the scientific study of evolution? Obviously the answer is nothing, if we speak of factual observation, but everything if we mean interpretation. I think it can be said that neither Kropotkin nor Huxley contributed much to the understanding of the mechanism of evolution by natural selection. The firm foundation for this was laid by the more sober work of Darwin and Wallace. After their work was done, neither one of these men concealed their mature and deeply moral views, but they did not derive them from the evidence for organic evolution. Darwin's views are well-known from the attitudes expressed in his letters. The last paragraph in Wallace's *Darwinism* (1889, page 478) is less widely known: "We thus find that the Darwinian theory, even when carried out to its extreme logical conclusion, not only does not oppose, but lends a decided support to, a belief in the spiritual nature of man. It shows us how man's body may have been developed from that of a lower animal form under the law of natural selection; but it also teaches us that we possess intellectual and moral faculties which could not have been so developed but must have had another origin; and for this origin we can only find an adequate cause in the unseen universe of Spirit". And only recently have I encountered for the first time (quoted by S. F. Mason in Centaurus 1953: Vol. 3, pp. 90–106) this sentence of Wallace published in 1900: "The only mode of natural selection that can act alike on physical, mental and moral qualities will come into play under a social system which gives equal opportunity of culture, leisure and happiness to every individual". This is a statement, not of fact, but of the same hope which motivated Kropotkin. The moral position of each man was an acquired character, influenced in each case by many factors beyond the facts of evolution accessible to both of them.

T. H. Huxley's position to which Kropotkin was so strongly opposed, was made perfectly clear in his Romanes lecture of 1893. "The ethical progress of society consists, not in imitating the cosmic process, still less in running away from it, but in combating it." It was Huxley's characterization of the cosmic (evolutionary) process as a "dog-eat-dog" affair against which Kropotkin's ire and opposition were aroused, and for which he tried to substitute his ethical idea of mutual aid, buttressed by evidence of cooperation in animal societies. Although T. H. Huxley occasionally justified his ethical views by his reasoning as a scientist, he did not try to derive them from evolutionary evidence. This remained for his grandson to do fifty years later (J. S. Huxley, Evolutionary Ethics, the Romanes Lecture, Oxford 1943); but the impression left by that attempt was that if one tries to derive today's ethics from evolutionary principles, he ends up by seeing its highest achievement in human societies of today and ascribing to the evolutionary process itself an ethical purpose. And this takes us out of the realm of science and into that unseen universe of belief into which Wallace, with more simplicity and directness, retreated.

These thoughts are not intended to belittle the achievements of Kropotkin or of Huxley or their followers in the last century or in this one. They faced our common dilemma and chose their sides; and since the processes of reason by which we all must live, are forwarded by defining alternatives and putting forward the issues to be clarified, we owe for this real service our thanks to all of those concerned in this controversy and to Professor Ashley Montagu for again calling it to our attention.

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LETTERS TO THE EDITORS

Correspondents alone are responsible for statements and opinions expressed. Letters are dated when received in the editorial office.

COMMENTS ON PROFESSOR LEWONTIN'S ARTICLE

The validity of the ideas and conclusions expressed by Professor Lewontin in his interesting and stimulating article appearing in the July issue of this Journal does not depend on the some-what novel definition he chooses to adopt of the term homeostasis; the definition may, however, lead to some confusion in comparisons of his ideas with those of others. Lewontin writes, in the section entitled *Definition of homeostasis*, "Operationally, then, one genotype is defined as being more homeostatic than another if its mean adaptive value is higher in the specified environmental range." And in the second section following he argues, in comparing two genotypes hypothesized for illustrative purposes, the one with the higher mean but more variable adaptive value is more homeostatic since "if homeostasis is to be any measure of the evolutionary future of a genotype, of the capacity of a genotype to adapt on the average to varying environment, no other result seems permissible."

While "adaptive value" and "homeostasis" seem almost equated by this definition and example, they do have the merit of forcefully directing attention to the role of variable environment. But, etymologically considered, homeostasis clearly and almost redundantly indicates constancy, and constancy is central in Cannon's use of the term. He has written (pages 23, 24, and 25 of *The Wisdom of the Body*, 1932): "The perfection of the process of holding a *stable state* in spite of extensive shifts of outer circumstance is not a special [illegible] bestowed upon the highest organisms but is the consequence of a gradual evolution. . . . The coordinated physiological processes which maintain most of the *steady states* in the organism are so complex and so peculiar to living beings—involving, as they may, the brain and nerves, the heart, lungs, kidneys and spleen, all working cooperatively—that I have suggested a special designation for these states, *homeostasis*. The word means a *condition*—a condition which may vary, but which is *relatively constant.*" [Most italics mine] It seems clear, here and in the remainder of his book, that homeostasis applies to particular *conditions* that are maintained in a *relatively stable state* by special mechanisms that have been evolved through natural selection; whereas the causative conditions, which are themselves variable, are called homeostatic devices or mechanisms (see for example chapter 11). As Cannon has used the term, homeostatic conditions seem important aids to, or components of, fitness, but not fitness itself; any particular organism, no matter how effective might be its homeostatic mechanisms, could conceivably possess flaws in other respects greatly reducing its fitness. Thus Cannon would not, I feel sure, consider homeostasis to be, as Lewontin assumes, a "measure of the evolutionary future of a genotype".

Lewontin's emphasis that natural selection sometimes favors variability as an adaptive character is timely and pertinent with respect to current discussions of homeostasis. As illustration he points to devices leading to homeostasis in body temperature of homoiotherms. Dilation of the peripheral blood vessels varies widely in response to changes in external temperature, a lability that adds to fitness. Cannon does not call degree of dilation a homeostatic character; since it is an aid to fitness, perhaps Lewontin does. Probably the divergence between Cannon's and Lewontin's definitions arises chiefly because the latter applies the term to organisms, whereas Cannon applies it to conditions in *an* organism.

Cannon also considered an extension of the homeostasis concept to conditions of stability in social institutions. It therefore seems modest and reasonable to apply the concept, as Lewontin and others have done, to certain aspects of morphology or functions that are determined in the course of embryogeny. Some such characters would, no doubt, tend to be greatly modified in their development by environmental vicissitudes were it not for special regulative mechanisms that have arisen through the agency of natural selection. Lewontin's discussion, however, brings clearly to view some of the difficulties in testing the hypothesis that homeostasis of developmental characters of this kind is favored by heterozygosis. It is perhaps harder to select appropriate characters for test, than to test them once selected.

Lerner's term genetic homeostasis, which is a further extension of the concept to populations and refers to their relative stability under continued selective breeding, may well be consonant with the spirit of Cannon's usage. The stabilities referred to by Lerner are active properties of the populations, not mere exhaustion of genetic variance; and the mechanisms responsible, although only vaguely understood at present, may well have developed, in part at least, through the influence of natural selection.

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March 9, 1956

A REPLY TO PROFESSOR DEMPSTER'S COMMENTS ON HOMEOSTASIS

Professor Dempster has been kind enough to comment on what he feels are some confusing points in my discussion of homeostasis. The major point of disagreement between us seems to be, whether the definition of homeostasis I have proposed is consonant with the original meaning of the concept as used by Cannon.

Cannon's thesis is summed up clearly by Dempster when he says, "homeostasis applies to particular conditions that are maintained in a *relatively stable state* by special mechanisms that have been evolved through natural selection; whereas the causative conditions, which are themselves variable, are called homeostatic devices or mechanisms". The problem arises when one tries to specify the "particular conditions that are maintained in a *relatively stable state*" as opposed to the "causative conditions, which are themselves variable". By what sign shall we know the conditions which are to be kept constant? The answer to this question lies in the reference to natural selection. If homeostatic mechanisms are established by natural selection it is because their establishment results in an increase in fitness of the system possessing them. The conditions which are maintained in a steady state are, then, those whose stability confers a greater degree of fitness. Conversely, any conditions or mechanisms whose variability increases fitness will also be selected and established. If one wishes to measure relative degrees of homeostasis by the stability or variability of some conditions or process, it is essential to know whether that stability or variability confers a greater degree of fitness on the system being studied. An increase in homeostasis, then, results in an increase in average fitness. If this were not the case, homeostasis could not be the product of natural selection. It is just because Cannon recognized homeostasis as a product of evolution, that only an adaptive constancy can be considered as evidence of homeostasis.

The question arises as to whether an increase in adaptive value is a necessary and sufficient criterion of an increase in homeostasis. Dempster believes not for he states that "any particular organism, no matter how effective its homeostatic mechanisms, could conceivably possess flaws in other respects greatly reducing its fitness." It is this proposition which lies at the base of our lack of agreement. In my view, the dichotomy between conditions of the organism which lie within the framework of its homeostatic adjustment, and those which lie without, does not exist. There is no aspect of the physiology or morphology of an organism which is not concerned in its homeostatic adjustment. Protoplasm is an unstable system. A constant input of energy is required just to maintain it, for when this input of energy fails, the system degenerates. Any surplus of energy, over and above that needed for simple maintenance is devoted to increase of the system through its own growth and by means of leaving progeny. The total resources of an organism are devoted to these two ends, which are the two components of fitness. Natural selection leads according to Cannon, to the establishment of a relation among the various physiological processes which will assure the maintenance of protoplasm under the widest variety of circumstances. We have extended this concept to include the other component of fitness, capacity to increase. With this extension, it is not possible to conceive of a physiological or morphogenetic process which is not intimately woven into the fabric of homeostatic adjustment.

If these arguments be granted, then an increase in homeostatic adjustment leads ineluctably to an increase in average fitness. Conversely an increase in *average* fitness can result only from greater homeostatic adjustment.

As a last point, Dempster raises the question of Lerner's "genetic homeostasis". We are in agreement that the tendency of a population not to change its genetic composition too suddenly in the face of new selective forces, may indeed be adaptive. The point should be made, however, that this resistance may also be disastrous. Again, conservatism *per se* is not homeostasis. It is only a conservatism with an adaptive consequence which can be regarded as homeostasis.

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TAXONOMIC CRITERIA IN AUSTRALIAN FROGS

It is of interest at times to examine a particular original paper in respect to its contained procedures, interpretations, and implications, especially when the paper is referred to in general argument. Accordingly, the following review has been occasioned by the citations of Brown and Wilson (1954) and Moore (1955) of work by the latter on Australian frogs as illustrative of reproductive incompatibility between populations without morphological differences.

The appositely named *Crinia insignifera* was described by Moore (1954) from individuals which would formerly have been called *Crinia signifera* on the basis of incompatible crosses between two males from near Perth, West Australia, and two females from Sydney, New South Wales. Twenty-one per cent of the embryos from one of these crosses developed into swimming larvae apparently capable of metamorphosis. A third West Australian male yielded sperm which fertilized the eggs of two Sydney females. These eggs developed into "nearly normal" embryos and "healthy" tadpoles, some of which "were kept until they underwent metamorphosis into seemingly normal frogs." Reciprocal crosses were not made, and in no case was the actual number of eggs or young mentioned.

From this evidence alone, Moore stated that "genetic divergence has reached a point where the two populations should be recognized as separate species." No constant morphological differences were found between the new species from southwestern Australia and the populations which retained the name *C. signifera*. It was noted, however, ther there is considerable geographic variation and local polymorphism. No indication was given of the nature of this variation nor were data presented to support the assertion of no constant morphological differences. No mention was made of the number of specimens compared.

There seems to be little published information on normal habits of *Crinia signifera*, but one might expect the breeding season in Western Australia to coincide with the rainy season, which is sharply delimited at Perth in contrast to the more even annual precipitation pattern at Sydney on the eastern side of the continent. If this supposition is correct, the type of the new species (and presumably the other West Australian males used in the experiments) was collected after or near the end of the normal breeding period. Since the dates and most of the conditions of the experiments were not given, the bearing this may have on the results obtained must be speculative.

Moore postulated that the range of *C. insignifera* was the southwestern segment of the continent, isolated by arid areas from that of *C. signifera,* which was restricted to northern West Australia east to Queensland and south to South Australia, Victoria, and Tasmania. His interpretation may be correct, but it seems based on far too little experimental data, the populations concerned not being adequately sampled, especially for a species showing considerable local polymorphism.

If the data do reflect natural conditions in these populations, they may be explained on the basis of differences correlated with the extent of geographic separation when the range was continuous. Such differences might be adaptive or simply due to distance (Wright, 1940). The present geographic isolation, which seems to furnish in part a reason for considering the two populations as distinct entities, poses the commonly occurring taxonomic question of degree of difference. Moore reported only partial incompatibility which, to us, may well mean conspecificity, considering the distance involved. It is perhpas significant in this connection that three crosses of Tasmanian and New South Wales frogs produced normal young only. Although the Tasmanian population has quite possibly been isolated from that of New South Wales for about the same length of time as has that of southwestern Australia (*cf.* Gentilli, 1949), the geographic separation in the former case is far less. In addition to confirmation of the present results, it would appear very pertinent to cross frogs from many other localities, and particularly some from northern West Australia with others from southwestern West Australia and from South Wales or Victoria.

The work reported in the same paper by Moore on the Australian *Hyla aurea* and *raniformis*, in which morphological differences between populations are known, is open to somewhat similar questions.

Thus, in view of the paucity of information on the morphological (and ecological) characters and on their geographic variations in *Crinia signifera* and *insignifera*, the results of *partial* incompatibility reported, the possible relationship between extent of separation and these results, and the lack of comparable cases of such species adequately demonstrated among other vertebrates, it would seem to behoove biologists to consider circumspectly the status of the new species of *Crinia*. The dangers in doing otherwise lie not so much in agreeing with Moore's interpretation in this particular case as in uncritically citing such work and in tacitly approving of the practices used in this instance for the establishment of vertebrate taxa, whether on "biological", ethological, or morphological grounds. The use of "biological" characters does not exempt a worker from consideration of *populations* and their characteristics.

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JAMES E. MOSIMANN

A REJOINDER TO RABB AND MOSIMANN'S LETTER

The questions raised by Rabb and Mosimann are of two sorts, namely, the interpretation of the data and the adequacy of the data. I will attempt to answer them in that order.

When certain frogs from Western Australia and New South Wales that belong to the same morphological species (*Crinia signifera*) are crossed, the hybrids show relatively specific types of defects. In different crosses the degree of defectiveness varies: in some crosses the developmental crises are passed and most of the embryos continue in their development; in other crosses most of the embryos die. I interpreted these data to indicate that two species are involved. The reason for so doing is that, in all the experiments on frog hybridization known to me (with the exception of the complex *Rana pipiens* situation where the taxonomic allocation of some geographic populations is a matter of arbitrary personal preference), such hybrid inviability is always associated with crosses of frogs belonging to unquestionably different morphological species. Rabb and Mosimann emphasize the "*partial* incompatibility which, to us, may well mean conspecificity...." Since it is impossible to obtain the most critical information to decide this point, namely, how the two populations of Crinia would behave if sympatric, I think the best we can do is to reason by analogy with what happens in crosses of other amphibians. If we do this the answer is reasonably clear: two species are involved.

So far as the adequacy of the data is concerned, I should first like to make one general observation. When defects are observed in the offspring from crosses of individuals of different populations of frogs, one is dealing with genetic differences of a considerable magnitude. These results are never encountered in crosses of individuals of the same species and sometimes not even in crosses of two morphological species. Furthermore, the results are usually so consistent that a few crosses are sufficient. In the four crosses of Western Australia males (of which there were three, not two as Rabb and Mosimann state) and New South Wales females that I was able to perform, the same type of defects were noticed in each, though the severity varied. The numbers of embryos used in the 4 crosses of New South Wales and Western Australia individuals were about equal in each and totalled 119 controls (N.S.W. × N.S.W.) and 183 hybrids.

Rabb and Mosimann are quite correct in surmising that my Western Australian males were collected near the end of the breeding season in Western Australia. However, the males were calling and they were collected at night in their breeding sites. This should ensure normal males. I used the males with confidence because in none of hundreds of crosses involving dozens of different amphibian species have I found the sperm of males collected in nature to be abnormal. Conceivably the sperm might be absent (though I have never observed this) and hence no fertilization could be expected. If sperm are present, and they certainly were in the males that I used, it is difficult to see how the time of their formation could have adversely affected their genetics.

The criticisms contained in the fifth paragraph of Rabb and Mosimann's letter are rather unfair since they belabor me for things I never said. So far as the individuals from Western Australia are concerned I wrote "Unless further experimentation proves it otherwise, we can regard all the frogs of south-west Australia, formerly known as *Crinia signifera*, as being *Crinia insignifera*." With respect to the other populations, I crossed individuals from central Tasmania, Kosciusko, and Sydney, New South Wales and, finding no cross-abnormalities, concluded on this evidence and on the absence of appreciable morphological differences, that a single species is involved. I had no material from Queensland, Northern Territory, or northern Western Australia and offered no verdict on the status of individuals from these states.

I cannot understand the criticism of paragraph six where Rabb and Mosimann write, "Such differences might be adaptive or simply due to distance." I fail to see how genetic differences can be due to distance. Presumably genetic differences are due to selection of mutations and possibly to genetic drift. Distance is not a cause of divergence though it may be associated with divergence. In any event 'differences are differences' and, if they serve as isolating mechanisms, it is immaterial how they arose.

There is a "paucity of information on the morphological (and ecological) characters and on their geographic variations" in my paper, as Rabb and Mosimann say. But my paper was not a monograph on the morphology and ecology of these frogs. These data are available and they will be presented in due time. Unless the two species were sympatric, data on any differences in ecology would be of limited value. So far as morphological characters are concerned my statement "I have not been able to find any constant morphological differences that will separate *Crinia insignifera* from *Crinia signifera* was based on approximately 20 individuals from Western Australia and more than a thousand living and preserved individuals from Eastern Australia. This lack of morphological differentiation between the eastern and western forms should not be unexpected by those familiar with the literature on these frogs. H. W. Parker had previously found no specific difference between eastern and western forms after a careful examination of more than 400 specimens. Parker's opinion was certainly independent of mine, it was obviously uninfluenced by any knowledge of the behavior of these forms when crossed, and hence is of greater value in reaching the conclusion of morphological identity. When a herpetologist of his competence is unable to find constant differences between the two populations, I think for all practical purposes we can assume they are the same morphological species. It matters not one bit if some future work establishes that the two differ morphologically in some slight though constant way. It is perfectly clear that they are different, though not in ways easily discernible with the superficial methods that are usually adequate in herpetological systematics.

Perhaps some of Rabb and Mosimann's misgivings arise from a misunderstanding of how final and complete I regard the opinions expressed in my paper. Let me say that I certainly do not consider the *Crinia signifera* question to be solved. These frogs present a variety of problems, some of which cannot be answered even with an endless amount of study of preserved material. I performed some experiments that can help to answer some of the questions, while at the same time they raised even more. Perhaps some day I shall be fortunate enough to return to the land where Crinia is found and try to answer a few more.

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April 24, 1956

JOHN A. MOORE

THE SIGNIFICANCE OF DIFFERENCES BETWEEN GENOTYPIC FREQUENCIES AND PHENOTYPIC FREQUENCIES

The comment by Sanghvi (1955) on my critique (Glass, 1954) of his data for seven genetical characters in endogamous groups in Bombay (Sanghvi and Khanolkar, 1952) is most interesting, and brings out strikingly the smallness of the effect of inbreeding on the gene frequencies even when the inbreeding coefficient is relatively high (.01). Of course, in small isolates such as the Dunker community in Franklin County, Pennsylvania, studied by Glass, Sacks, Jahn, and Hess (1952), the coefficient of inbreeding may be considerably higher, and one must keep in mind the generality of isolates of all existing sizes. In the Dunker community, for example, *F* (Wright's coefficient of inbreeding) was estimated to be .0254 and might be higher. Yet even here, for a gene frequency of .50, the percentage of the recessive homozygotes would amount only to 25.63% instead of 25.0%. It is therefore quite true, as Sanghvi has pointed out, that in the case of common alleles the difference is of no great significance

I regret that Sanghvi's note was not brought to my attention until after its publication, as otherwise I might have discovered sooner the error which led me to state (Glass, 1954) that the statistical significance of the differences between the MN phenotypic frequencies in different endogamous groups was reduced when one converted the data into genotypic frequencies. Checking my original calculations, I find that in shifting from phenotypic frequencies to genotypic frequencies I failed to double the sample size. Sanghvi is therefore quite correct about the increased, rather than decreased, significance which results when the conversion is made.

This leads to the rather amusing conclusion that Sanghvi has supported my original point, namely, that genotypic rather than phenotypic frequencies ought to be used whenever possible, with a better reason than the one I advanced myself. The loss of a degree of freedom in making the conversion may bring out statistically significant differences that are obscured in the crude data.

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June 20, 1956

DATURA RESEARCH MATERIAL AVAILABLE

The Smith College Genetics Experiment Station was discontinued at the end of 1955, a year after the death of Dr. Albert F. Blakeslee, its founder and director since 1942. For many years the investigations of Dr. Blakeslee and his colleagues at Smith College, and earlier at the Department of Genetics of the Carnegie Institution of Washington, had resulted in the announcement of many new facts and principles resulting from new chromosomal varieties of Datura with duplicated chromosomes or chromosome sections. However, there are many unfinished research problems and opportunities for investigations with Datura in genetics, cytogenetics, plant physiology and morphology. The ten herbaceous species of Datura provide an excellent opportunity to study many angles of the species problem: hybridization, compatible and incompatible crosses, the barriers to crossability. Tetraploid, as well as diploid lines of most species as well as chromosomally different races are available. In *Datura stramonium* there are available many $2n + 1$ types, nearly a hundred different chromosomal races (Prime types), as well as many gene types. Some races have been maintained by selfing for more than forty generations. The National Science Foundation has recently made a grant to Smith College to finance the assembling of the data on Datura genetics and related problems, and its publication in a monograph on Datura. This will be in charge of Amos G. Avery, and the principal contributors will be Dr. Sophie Satin, and Dr. J. Rietsema.

Datura is almost an ideal plant for research. It is easy to grow either in the greenhouse or field; selfing and crossing is simple and a large number of seed is produced. As many as four generations a year have been obtained in special instances although two or three are usual. It is also readily propagated by cuttings or grafting.

Seed of all the species and of most of the races and types have been grown and placed in controlled cold storage. These are available to any investigator who desires to continue some phase of the Datura research. Application should be made to Dr. H. H. Plough, Amherst College, Amherst, Massachusetts indicating the seeds desired and the general nature of the investigation planned. Seeds will be mailed promptly.

Committee of the Genetics Society
of America for the Preservation of
A. F. Blakeslee's Datura material:

H. H. PLOUGH, *Chairman*
R. E. CLELAND
M. DEMEREC
P. C. MANGELSDORF
E. W. SINNOTT

DEPARTMENT OF BIOLOGY
AMHERST COLLEGE
AMHERST, MASS.
May 31, 1956

INDEX TO VOL. XC

Alphabetic Table of Contents by Authors

ACTON, A. B., Crossing over within inverted regions in Chironomus, 63

AMERICAN SOCIETY OF NATURALISTS, address of president, 145; reports, 139

BARDACH, JOHN E., The sensitivity of the goldfish (*Carassius auratus* L.) to point heat stimulation, 309

BEACH, FRANK A., and JULIAN JAYNES, Studies on maternal retrieving in rats: II. Effects of practice and previous parturitions, 103

BENNETT, J. H., Modes of tetrad formation, 195

BLOCH, ROBERT, book notices, 144, 205

BRAUN, ARMIN C., A study on the nature of autonomy in neoplastic plant cells, 227

BROWER, LINCOLN P., and JANE VAN-ZANDT BROWER, Cryptic coloration in the Anthophilous moth *Rhododipsa masoni*, 177

CAIN, A. J., and P. M. SHEPPARD, Adaptive and selective value, 202

DEMPSTER, EVERETT R., Comments on Professor Lewontin's article, 385

DEXTER, RALPH W., The early *American Naturalist* as revealed by letters to the founders, 209

DOBZHANSKY, THEODOSIUS, What is an adaptive trait?, 337

DUNN, L. C., Mutual aid and evolution (book review), 381

FELL, HONOR B., Comments on Dr. White's article on tissue culture, 61

FROTA-PESSOA, O., A mutant in *Drosophila pseudoobscura* which alters the pigmentation of the testicular envelope without changing that of the eyes, 133

GARBER, E. D., A Nutrition-inhibition hypothesis of pathogenicity, 183

GLASS, BENTLEY, The significance of differences between genotypic frequencies and phenotypic frequencies, 395

GRANT, VERNE, The influence of breeding habit on the outcome of natural hybridization in plants, 319

GROSCH, DANIEL S., Lethality induced by feeding radiophosphorus to male Habrobracon, 200

GUNTER, GORDON, A description of a red drum, *Sciaenops* ocellata, without eyes, with a consideration of the implications, 267

HYMAN, L. H., book notice, 335

JACOBS, WILLIAM P., Internal factors controlling cell differentiation in the flowering plants, 163

KENWORTHY, WALTER, The effect of oxygen concentration on the dose-action survival curves for irradiated Habrobracon eggs, 119

KHISHIN, AZIZ F., Induction of mutations by formaldehyde solutions in *Drosophila melanogaster*, 377

KIMBALL, A. W., Approximate confidence intervals for specific locus mutation rates, 369

KOREF, SUSI E., Melanotic tumors in Drosophila as possible phenodeviants, 270

KUSNEZOV, N. N., A comparative study of ants in desert regions of Central Asia and of South America, 349

LEVINE, R. P., Crossing over and inversions in coadapted systems, 41

LEWIN, RALPH A., Problems of sex in unicellular algae, 331

LEWONTIN, R. C., Studies on homeostasis and heterozygosity. I. Abdominal bristle number in second chromosome homozygotes of *Drosophila melanogaster*, 237; A reply to Professor Dempster's Comments on homeostasis, 387

MOORE, JOHN A., A rejoinder to Rabb and Mosimann's letter, 391; book notice, 333

NANNEY, DAVID L., Caryonidal inheritance and nuclear differentiation, 291

OSTER, I. I., and F. H. SOBELS, "Natural implantation" of a lethal mutation in *Drosophila melanogaster*, 55

PLOUGH, H. H., Datura research material available, 394

PUBLICATIONS RECEIVED, 143, 205, 333

RABB, GEORGE B. and JAMES E. MOSIMANN, Taxonomic criteria in Australian frogs, 389

RENSCH, BERNARD, Increase of learning capability with increase of brain-size, 81

ROBINSON, H. F., R. E. COMSTOCK, A. KHALIL, and P. H. HARVEY, Dominance versus over-dominance in heterosis: Evidence from crosses between open-pollinated variaties of maize, 127

RUSSELL, W. L., Comparison of X-ray-induced mutation rates in Drosophila and mice, 67

SCHALET, ABRAHAM, A second case of translocation between paternal and maternal chromosomes, 329

SCHWARTZ, DREW, and CLELLA E. BAY, Further studies on the reversal in the seedling height dose curve at very high levels of ionizing radiations, 323

SMITH, STANLEY G., Cytotaxonomy of Curculionidae (Coleoptera), 137

STANLEY-JONES, D., Axial rotation and the origin of vertebrates, 361

STEPHENS, S. G., The composition of an open pollinated segregating cotton population, 25

STRAW, RICHARD M., Floral isolation in Penstemon, 47

THIMANN, KENNETH V., Twin themes of physiology, 145
TRINKAUS, J. P., The differentiation of tissue cells, 273
WHITAKER, THOMAS W., The origin of the cultivated Cucurbita, 171
WHITING, P. W., and MARION KAYHART, Mutations at a single locus in the wasp Mormoniella, 111
WRIGHT, SEWALL, Modes of selection, 5

YARWOOD, C. E., Generation time and the biological nature of viruses, 97

ZWILLING, EDGAR, Reciprocal dependence of ectoderm and mesoderm during chick embryo limb development, 257

Subject Index

adaptations, 13
adaptive traits, 337
adaptive value, 202, 385, 387
American Naturalist, history of, 209
American Soc. Nat.
  Secys report, 139
  president's address, 145
  symposium, 163, 227
auxin, 148, 163, 231

brain size, and learning, 81

Carassius auratus, 309
caryonidal inheritance, 291
Cavia cutleri, 9
chick embryo, limb development, 257
chironomus species, 63
confidence intervals, 369
conjugation,
  in ciliates, 292
cotton, 25
Crinia signifera, 389, 391
Crinia insignifera, 389, 391
crossing over
  and inversions, 41
  in chironomus, 63
crown gall, 228
cryptic coloration, 177
Cucurbita,
  origin of, 171
Curculionidae cytotaxonomy, 137
cytotaxonomy, 137

Datura, research materials, 394
development,
  of limbs, 258
differentiation,
  in plants, 163
  in tissue cells, 273
  nuclear, 291
dominance
  and heterosis, 127
Drosophila, 69
  willistoni, 337
  paulistorum, 337
  equinoxialis, 337
  tropicalis, 337
  melanogaster, 55, 237, 270, 329, 377
  pseudobscura, 41, 133

eggs,
  effects of radiation, 119
elephant,
  learning in, 89
evolution,
  of behavior, 91

formaldehyde as mutagen, 377

generation time
  of viruses, 97
genes and development, 343
gene frequencies, 37
Gilia species, 319
goldfish, heat sensitivity, 309
guinea pig, 9

Habrobracon
  effect of oxygen, 119
  effect of radiophosphorus, 200
heterosis,
  in maize, 127
homeostasis, 237, 346, 385, 387
hybridization, in plants, 319
Hylobius species, 137

inhibition,
  in plant physiology, 145
  and pathogenicity, 183, 190
introgressive hybridization, 21
isolating mechanisms, 51

learning capacity, 81
lethality,
  in Habrobracon, 200
limb development
  in chick embryo, 257

maize,
  heterosis, 127
  effect of radiation, 323
maternal behavior, 103
Mormoniella, 111
mouse,
  mutation rates, 69
  behavior, 88
  reaction to pathogens, 185
mutants,
  in Drosophila, 133
  in chicken, 260
mutation,
  induction of, 111, 377
  rates of, 69, 369
  macro, 20
  lethal, 55, 111

Neoplastic
  in plants, 227
nucleus, differentiation, 291, 304
nutrition,
  and pathogenicity, 183

overdominance,
  in maize, 127
oxygen,
  effects on egg survival, 119

Paramecium aurelia, 291
parasitism, 191
Penstemon,
  floral isolation in, 47
phenodeviants,
  in Drosophila, 270

physiology,
  promotion and inhibition, 145
plant hormones, 163, 231
plant neoplastics, 227
plant, hybridization, 319
pleiotropism, 6, 342
pollinating agents, 48
polyploidy,
  in plants, 321

radiation,
  and mutation, 69, 111, 329
  and oxygen, 119
  and growth, 323
radiophosphorus
  and lethality, 200
rat,
  behavior, 88, 103
Red drum,
  eyeless, 267
Rhododipsa masoni, 177

Scianops ocellata
  eyeless, 267
selection, 5, 202
  interdeme, 16
selective value, 202
sex, in algae, 331
sibling species, 337
synergism
  and inhibition, 159

taxonomic criteria, 389
tetrad formation, 195
Tetrahymena pyriformis, 291
tissue culture, 61, 274
translocations,
  in Drosophila, 329
tumors,
  in plants, 227
  in Drosophila, 270

virus,
  generation time, 97

wound healing, 235

RECORDS

# of the

AMERICAN SOCIETY OF NATURALISTS

Volume V
Part Two

EXECUTIVE COMMITTEE, 1956

Officers

	Years to Serve
Dr. E. Newton Harvey, President *Dept. of Biology, Princeton University*	1956
Dr. Frank A. Brown, Jr., Vice-President *Dept. of Zoology, Northwestern University*	1956
Dr. Bruce Wallace, Secretary *Biological Laboratory, Cold Spring Harbor, N. Y.*	1956–1958
Dr. Carl P. Swanson, Treasurer *Dept. of Biology, Johns Hopkins University*	1954–1956

Additional Members of the Executive Committee

Dr. Kenneth V. Thimann *Biological Laboratories, Harvard University*	1956–1958
Dr. M. Demerec *Carnegie Inst. of Wash., Cold Spring Harbor, N. Y.*	1956–1957
Dr. Elmer G. Butler *Dept. of Biology, Princeton University*	1956
Dr. Warren P. Spencer *Dept. of Biology, College of Wooster*	1956

MEETINGS AND PRESIDENTS OF THE AMERICAN SOCIETY OF NATURALISTS

1.	1883.	April 10–11	Springfield	Alpheus Hyatt,	Chair
2.	1883.	Dec. 27–28	New York	Alpheus Hyatt,	President
3.	1884.	Dec. 29–30	Washington	Alpheus Hyatt,	"
4.	1885.	Dec. 29–30	Boston	Grove K. Gilbert,	"
5.	1886.	Dec. 28–30	Philadelphia	Grove K. Gilbert,	"
6.	1887.	Dec. 27–29	New Haven	Harrison Allen,	"
7.	1888.	Dec. 27–28	Baltimore	Harrison Allen,	"
8.	1889.	Dec. 27–28	New York	George L. Goodale,	"
9.	1890.	Dec. 31	Boston	H. Newell Martin,	"
10.	1891.	Dec. 29–30	Philadelphia	William North Rice,	"
11.	1892.	Dec. 28–29	Princeton	Henry F. Osborn,	"
12.	1893.	Dec. 27–28	New Haven	R. H. Chittenden,	"
13.	1894.	Dec. 27–28	Baltimore	Charles S. Minot,	"
14.	1895.	Dec. 26–27	Philadelphia	Edward D. Cope,	"
15.	1896.	Dec. 29–30	Boston	William B. Scott,	"
16.	1897.	Dec. 28–29	Ithaca	Charles O. Whitman,	"
17.	1898.	Dec. 29	New York	Henry P. Bowditch,	"
18.	1899.	Dec. 28	New Haven	William G. Farlow,	"
19.	1900.	Dec. 27–28	Baltimore	Edmund B. Wilson,	"
20.	1901.	Dec. 31–Jan. 1	Chicago	William T. Sedgwick,	"
21.	1902.	Dec. 30–31	Washington	J. McKeen Cattell,	"
22.	1903.	Dec. 29–30	St. Louis	William Trelease,	"
23.	1904.	Dec. 27–28	Philadelphia	Edward L. Mark,	"
24.	1906.	Dec. 28	New York	Charles B. Davenport,	"
25.	1907.	Dec. 31	Chicago	James P. McMurrich,	"
26.	1908.	Dec. 31	Baltimore	David P. Penhallow,	"
27.	1909.	Dec. 29	Boston	Thomas H. Morgan,	"
28.	1910.	Dec. 29–30	Ithaca	Daniel T. MacDougal,	"
29.	1911.	Dec. 28	Princeton	Herbert S. Jennings,	"
30.	1913.	Jan. 2	Cleveland	Edwin G. Conklin,	"
31.	1913.	Dec. 31	Philadelphia	Ross G. Harrison,	"
32.	1914.	Dec. 31	Philadelphia	Samuel F. Clarke,	"
33.	1915.	Dec. 30	Columbus	Frank R. Lillie,	"
34.	1916.	Dec. 29–30	New York	Raymond Pearl,	"
35.	1918.	Jan. 1	Pittsburgh	George H. Shull,	"
36.	1918.	Dec. 28	Baltimore	William E. Castle,	"
37.	1919.	Dec. 30–31	Princeton	Edward M. East,	"
38.	1920.	Dec. 30–31	Chicago	Jacques Loeb,	"
39.	1921.	Dec. 29	Toronto	Bradley M. Davis,	"
40.	1922.	Dec. 29	Boston	Wm. H. Wheeler,	"
41.	1923.	Dec. 29	Cincinnati	R. A. Emerson,	"
42.	1925.	Jan. 1	Washington	Wm. H. Howell,	"
43.	1925.	Dec. 28–30	New Haven	C. Hart Merriam,	"
44.	1926.	Dec. 29	Philadelphia	J. Arthur Harris,	"
45.	1927.	Dec. 30	Nashville	C. E. McClung,	"
46.	1928.	Dec. 29	New York	H. H. Donaldson,	"
47.	1930.	Jan. 1	Des Moines	G. H. Parker,	"
48.	1931.	Jan. 1	Cleveland	A. F. Blakeslee,	"
49.	1931.	Dec. 30	New Orleans	S. J. Holmes,	"
50.	1932.	Dec. 30	Atlantic City	R. A. Gortner,	"
51.	1933.	Dec. 30	Boston	B. E. Livingston,	"
52.	1934.	Dec. 29	Pittsburgh	A. F. Shull,	"
53.	1936.	Jan. 2	St. Louis	John C. Merriam,	"
54.	1936.	Dec. 31	Atlantic City	C. E. Allen,	"
55.	1937.	Dec. 30	Indianapolis	D. H. Tennent,	"
56.	1938.	Dec. 30	Richmond	R. M. Yerkes,	"
57.	1939.	Dec. 30	Columbus	I. F. Lewis,	"
58.	1941.	Jan. 1	Philadelphia	J. H. Bodine,	"
59.	1941.	Dec. 30	Dallas	W. W. Cort.	"
60.	1942.		No Meeting	R. E. Cleland,	"
61.	1943.		No Meeting	H. J. Muller,	"
62.	1944.	Sept. 14	Cleveland	Fay-Cooper Cole,	"
63.	1945.	Mar. 30, 1946	St. Louis	Edmund W. Sinnott,	"
64.	1946.	Dec. 31	Boston	Charles W. Metz,	"
65.	1947.	Dec. 31	Chicago	K. S. Lashley,	"
66.	1948.	Sept. 13	Washington, D.C.	P. R. Burkholder,	"
67.	1949.	Dec. 29	New York	T. M. Sonneborn,	"
68.	1950.	Sept. 13	Columbus	Th. Dobzhansky,	"
69.	1951.	Sept. 12	Minneapolis	P. C. Mangelsdorf,	"
70.	1952.	Sept. 10	Ithaca	Sewall Wright,	"
71.	1953.	Dec. 28	Boston	L. J. Stadler,	"
72.	1954.	Sept. 7	Gainesville	M. Demerec,	"
73.	1955.	Sept. 7	East Lansing	K. V. Thimann	"

VICE-PRESIDENTS

H. Newell Martin, 1883-85
Alpheus S. Packard, 1883-84
Edward D. Cope, 1885-87
Harrison Allen, 1885-86
George L. Goodale, 1886-88
H. S. Williams, 1887-89, 1898, 1900
Henry F. Osborn, 1888-89
G. Brown Goode, 1889
Samuel F. Clarke, 1890-93
William North Rice, 1890
R. Ramsey Wright, 1890
T. Wesley Mills, 1891
Charles O. Whitman, 1891, 1895-96
George Baur, 1892-93
William H. Dall, 1892-94
William Libbey, 1893-95
Sidney I. Smith, 1894
William G. Farlow, 1895-96
Theodore Gill, 1896
Henry P. Bowditch, 1897
Edmund B. Wilson, 1897
William P. Wilson, 1897
William James, 1898
Simon H. Gage, 1898
Hermon C. Bumpus, 1899
William H. Howell, 1899, 1909
Frederic H. Gerrish, 1899
Roland Thaxter, 1900
Franklin P. Mall, 1900
Leland O. Howard, 1901-02
Lucien M. Underwood, 1901
J. Walter Fewkes, 1901
Charles D. Walcott, 1902
David P. Penhallow, 1902, 1907
Franz Boas, 1903-04
John M. Coulter, 1903-04
Charles B. Davenport, 1906
Henry H. Donaldson, 1906
Thomas H. Morgan, 1908
Robert A. Harper, 1908-09
Herbert S. Jennings, 1910
George H. Shull, 1911
Ross G. Harrison, 1912
Edward M. East, 1913
Frank R. Lillie, 1914
Rollins A. Emerson, 1915
Albert F. Blakeslee, 1916
Leon J. Cole, 1917
Guy N. Collins, 1918
John H. Gerould, 1919
Bradley M. Davis, 1920
Henry E. Crampton, 1921
A. H. Sturtevant, 1922
Lorande Loss Woodruff, 1923
Lester W. Sharp, 1924
Warren H. Lewis, 1925
John A. Detlefsen, 1926
Donald F. Jones, 1927
Benjamin M. Duggar, 1928
A. Franklin Shull, 1929
Charles Zeleny, 1930
E. J. Kraus, 1931
H. J. Muller, 1932
E. V. Cowdry, 1933
E. B. Babcock, 1934
F. E. Lutz, 1935
Wm. K. Gregory, 1936
Ivey F. Lewis, 1937
J. H. Bodine, 1938
W. H. Taliaferro, 1939
Karl Sax, 1940
L. H. Snyder, 1941
L. C. Dunn, 1942
B. M. Duggar, 1943
Charles W. Metz, 1944
Karl S. Lashley, 1945
Herman A. Spoehr, 1946
M. Demerec, 1947
T. M. Sonneborn, 1948
K. V. Thimann, 1949
Harold H. Plough, 1950
B. F. Kaufmann, 1951
C. B. Van Niel, 1952
Alexander Hollaender, 1953
Ralph W. Chaney, 1954
E. G. Butler, 1955

SECRETARIES

Samuel F. Clarke, 1883, 1886-88
Charles Sedgwick Minot, 1884-85
Henry H. Donaldson, 1889-91
Thomas H. Morgan, 1892-93, 1899
William A. Setchell, 1894
Hermon C. Bumpus, 1895-98
Albert D. Mead, 1900-01
Ross G. Harrison, 1902-03
Charles B. Davenport, 1904-05
William E. Castle, 1906
William J. Moenkhaus, 1906
Edward L. Thorndike, 1907
Thomas G. Lee, 1907-09
Henry McE. Knower, 1908-09
Charles R. Stockard, 1910-11
Aaron L. Treadwell, 1912
Bradley M. Davis, 1913-19
A. Franklin Shull, 1920-26
Leon J. Cole, 1927-31
E. W. Lindstrom, 1932-34
A. M. Banta, 1935-37
Ralph E. Cleland, 1938-40
Alfred C. Kinsey, 1941-43
Wm. Randolph Taylor, 1944-46
W. S. Stone, 1947-49
Bentley Glass, 1950-52
Warren P. Spencer, 1953-1955

TREASURERS

William B. Scott, 1883-84
Charles A. Ashburner, 1885-86
William T. Sedgwick, 1887-93
Edward G. Gardiner, 1894-95
John B. Smith, 1896-99
Maynard M. Metcalf, 1900-02
Bashford Dean, 1903
Hermann Von Schrenk, 1904-09
Edward M. East, 1910-11
William E. Kellicott, 1912
J. Arthur Harris, 1913-25
Edmund W. Sinnott, 1926-28
Sewell Wright, 1929-32
M. Demerec, 1933-35
R. A. Brink, 1936-38
H. H. Plough, 1939-41
W. R. Irwin, 1942-44
T. M. Sonneborn, 1945-47
D. P. Costello, 1948-50
D. F. Poulson, 1951-53
Carl P. Swanson, 1954-1956

THE ORIGINAL CALL

The following is a copy of the original call for the first meeting, which was issued by Professor Samuel F. Clarke, of Williamstown, in March, 1883:

> A number of American workers in Biology, desiring to have established an association of American naturalists for business purposes, extend to you a cordial invitation to join in a movement looking toward that end.
>
> The intention is to have an annual meeting, for the purpose of discussing topics of common interest, for which, at present, no opportunity is afforded; as, for example, museum interests, in connection with which each museum director could indicate his plan of work, the special group of which he was making exhaustive collections, so that work may not be uselessly duplicated in many places; methods of museum work; methods of exhibition, etc.; methods of laboratory work; laboratory technique; new and valuable points in staining, mounting, cutting, and preserving of sections; systems of instruction in various departments of natural science; methods with small elective classes; with large college classes; the position which the observational sciences should hold in the college curriculum; the amount of natural science which should appear in college entrance examinations; the amount and character of such instruction in preparatory school, etc.
>
> It is further believed that such a society could materially influence for the better the cause of science in America; that it would have a very healthful general effect, and could exert a strong influence in many directions where at present it seems to be very much needed. It is proposed to have the first meeting, which will be preliminary to organization, and, therefore, of prime importance, at Springfield, Mass., in the Springfield High School Hall, Friday, April 10.
>
> The following gentlemen are interested in the enterprise, and nearly all of them will be among those present: [the names are J. M. Tyler, A. Hyatt, C. S. Minot, A. S. Packard, Jr., S. H. Scudder, H. N. Martin, W. T. Sedgwick, G. Macloskie, W. Libbey, H. F. Osborn, W. B. Scott, W. N. Rice, S. I. Smith, S. F. Clarke (secretary)].

THE CONSTITUTION

ARTICLE I

Name and Objects

Section 1. This association shall be known as the American Society of Naturalists,

Section 2. The object of this Society shall be the association of working naturalists for the discussion, advancement and diffusion of knowledge concerning the broader biological problems, including organic evolution, thus serving to correlate the various biological sciences into a common philosophy of biology.

ARTICLE II

Members

Section 1. Membership in the Society shall be limited to persons professionally engaged in some branch of natural history, as, instructors in natural history, officers of museums and other scientific institutions, physicians, and others, who have essentially promoted the natural history sciences by original contributions of any kind. Any member may present to the executive committee of the Society, through the secretary, names of candidates for membership and those candidates who are approved by the committee may be elected to membership in the Society by a majority of the members present at any meeting of the Society. The names of candidates not elected to membership within three years of the date of consideration shall be removed from the list of nominees unless renominated.

Section 2. Each active member shall pay to the treasurer of the Society annual dues of the amount provided in the By-Laws, and considered due on January first of each year. The name of any member two years in arrears for annual assessments shall be erased from the list of the Society, and such person can only regain membership by re-election.

Section 3. Honorary members, exempt from the payment of dues, may be elected upon the recommendation of the executive committee by a two-thirds vote of the members present at any meeting of the Society. The number of honorary members is limited to ten. Emeritus members, upon retirement from their professional posts, are exempt from dues.

ARTICLE III

Officers

Section 1. The officers of the Society shall be a president, a vice-president, a secretary and a treasurer. These, together with the three most recent past-presidents and the retiring vice-president, shall constitute the executive committee of the Society.

Section 2. The president and vice-president shall be elected for a term of one year, the secretary and treasurer for a term of three years. Each president on retirement shall serve on the executive committee for three years. Each vice-president and secretary on retirement shall serve on the executive committee for one year. The election of officers shall take place at the annual meeting of the Society, and their official terms shall begin on the following January first. They shall continue in office until new officers are installed. In case the annual meeting is postponed to a date subsequent to January first of the next year, the terms of the new officers shall begin immediately upon election.

Section 3. The officers named in Section 1 shall discharge the duties usually assigned to these respective officers. The executive committee shall recommend to the Society from time to time such measures as they may deem expedient for the purposes of the Society, besides discharging the specific duties assigned to them by this constitution.

Section 4. Vacancies in the board of officers, occurring by death, resignation, or otherwise, may be filled by election by ballot at any meeting of the Society. A vacancy in the secretaryship or treasurership occurring in the interval of the meetings of the Society may be filled by appointment by the executive committee; but the person so appointed shall hold office only until the next meeting of the Society.

ARTICLE IV

Meetings

Section 1. The annual meeting shall be held during convocation week, unless otherwise ordered by the executive committee.

Section 2. Special meetings may be appointed at any time by a vote of the Society or of the executive committee.

Section 3. Sections of the Society may be organized in any locality, with the approval of the Society in each case, by ten or more members for the purpose of holding meetings for the presentation of scientific papers. Such sections shall have the right to elect their own officers and associate members, but associate membership in any section shall not confer membership in the Society.

ARTICLE V

Quorum

Fifteen members shall constitute a quorum of the Society, and three a quorum of the executive committee.

ARTICLE VI

Accounts

A committee shall be appointed at each annual meeting to audit the accounts of the treasurer for the year closing with that meeting.

ARTICLE VII

Affiliated Societies

The Society may affiliate with other scientific organizations.

ARTICLE VIII

By-Laws

Section 1. By-laws recommended by the executive committee may be adopted at any meeting by a majority vote.

Section 2. By-laws may be repealed at any meeting upon recommendation of the executive committee, by a majority vote.

ARTICLE IX

Amendments

Amendments to the constitution, recommended by the executive committee, may be adopted at any annual meeting by a vote of two-thirds of the members present.

BY-LAWS

1. The programs shall be arranged by the vice-president in consultation with the president and the secretary.

2. Each president on retiring shall appoint a nominating committee of three members, including a chairman, for officers to be elected at the next annual meeting. The retiring president and the secretary shall be ex-officio members of this committee.

3. The Executive Committee shall be empowered to appoint an Editor for *The American Naturalist*, to serve for a five year term. The Executive Committee, in consultation with the Editor, shall appoint an Editorial Board to advise the Editor in matters of policy. Each member of the Editorial Board shall serve for a term of three years.

4. In return for $3.50 per individual subscription, the Science Press will provide an annual subscription to *The American Naturalist* for each active member not in arrears for dues; and also for each emeritus member who elects to subscribe for $3.50 per annum.

5. The Records of the Society shall be published once every three years beginning in 1914. The Records shall contain the constitution and by-laws of the Society, the minutes of all meetings held within the period covered, the treasurer's reports and a full list of members of the Society.

6. The Society shall reimburse the secretary for traveling and hotel expenses incurred in attending the annual meeting.

7. Active members shall pay dues of $5.00, which shall include a subscription to *The American Naturalist*. Emeritus members may receive the journal upon payment of $3.50 annually.

In the library of the Wistar Institute of Philadelphia will be found a complete set of the Records of the American Society of Naturalists together with printed announcements, programs, and reports, and also certain correspondence of historical interest. This matter, the property of the Society, is cared for through the courtesy of the Wistar Institute.

Official Publications of the Society

RECORDS		
Volume I.	Parts 1-12.	1884-1895.
Volume II.	Parts 1-10.	1896-1911.
Volume III.	Parts 1-6.	1912-1931.
Volume IV.	Part 1.	1932-1934.
Volume IV.	Part 2.	1935-1937.
Volume IV.	Part 3.	1938-1940.
Volume IV.	Part 4.	1941-1943.
Volume IV.	Part 5.	1944-1946.
Volume IV.	Part 6.	1947-1949.
THE AMERICAN NATURALIST		
Volume LXXXV	Nos. 820-825	1951.
Volume LXXXVI	Nos. 826-831	1952.
Volume LXXXVII	Nos. 832-	1953.
	(including Records, V, Part 1)	
THE AMERICAN NATURALIST		
Volume LXXXVIII	Nos. 838-843	1954.
Volume LXXXIX	Nos. 844-849	1955.
Volume XC	Nos. 850-	
	(including Records, V, Part 2)	

MEMBERSHIP LIST

The date indicates the meeting at which the member was elected; thus 1929 denotes the meeting for the year 1929, although that meeting was actually held on January 1, 1930.

HONORARY MEMBERS

DUGGAR, BENJAMIN M., Ph.D., Sc.D. (hon.), LL.D., Lederle Lab., Pearl River, N. Y. 1915.

GOLDSCHMIDT, RICHARD B., Ph.D., M.D., Sc.D., Professor Emeritus of Zoology, *University of California*, Berkeley 4, Calif. 1914.

HARRISON, ROSS G., Ph.D., Sc.D., LL.D., Professor Emeritus of Biology, Osborn Zoological Lab., *Yale University*, New Haven 11, Conn. 1893.

PATTERSON, JOHN THOMAS, Ph.D., D.Sc. (hon.), Professor Emeritus of Zoology, *University of Texas*, Austin 12, Tex. 1919.

PAYNE, FERNANDUS, Ph.D., Sc.D. (hon.), LL.D., Professor Emeritus of Zoology, *Indiana University*, Bloomington, Ind. 1911.

SHULL, A. FRANKLIN, Ph.D., Professor Emeritus of Zoology, *University of Michigan*, 431 Highland Road, Ann Arbor, Mich. 1911.

WRIGHT, SEWALL, Sc.D., Sc.D. (hon.), LL.D., L. J. Cole Professor of Genetics, *University of Wisconsin*, Madison 6, Wis. 1915.

EMERITUS MEMBERS

ABRAMS, LEROY R., Ph.D., Professor of Botany, Emeritus, 653 Cabrillo, *Stanford University*, Calif. 1943.

ACKERT, JAMES E., Ph.D., Emeritus Dean, Graduate School, *Kansas State College*, Manhattan, Kan. 1931.

ADAMS, CHARLES C., Ph.D., Sc.D., Retired Director, *New York State Museum*, 149 Manning Blvd., Albany 3, N.Y. 1904.

ADDISON, WILLIAM H. F., M.D., 286 East Sidney Ave., Mount Vernon N.Y. 1920.

ALLARD, H. A., B.S., 3000 7th St., North, Arlington, Va. 1931.

ALLEN, ARTHUR A., Ph.D., Dept. of Conservation, *Cornell University*, Ithaca, N.Y. 1934.

ANDREWS, ETHAN A., Ph.D., Professor Emeritus, Dept. of Biology, *Johns Hopkins University*, Baltimore, Md. 1888.

APPLEMAN, CHARLES O., Ph.D., Emeritus Dean, Graduate School, *University of Maryland*, College Park, Md. 1925.

BAILEY, C. H., Ph.D., Dept. of Agriculture, *University of Minnesota*, University Farm, St. Paul, Minn. 1933.

BAILEY, IRVING W., M.F., D.Sc., Herbarium, 22 Divinity Ave., *Harvard University*, Cambridge 38, Mass. 1913.

BAITSELL, GEORGE A., Ph.D., Professor Emeritus of Biology, Osborn Zoological Laboratory, *Yale University*, New Haven, Conn. 1915.

BARSS, HOWARD P., S.M., Office of Experiment Stations, U.S. Dept. of Agriculture, Washington 25, D.C. 1936.

BARTLETT, HARLEY HARRIS, A.B., Dept. of Botany, *University of Michigan*, Ann Arbor, Mich. 1913.

BARTSCH, PAUL, Ph.D., D.Sc., Professor Emeritus, *George Washington University*, 'Lebanon', Lorton, Va. 1931.

BEAL, JOHN M., Ph.D., Professor Emeritus of Botany, *University of Chicago*, Chicago 37, Ill. 1939.

BEEBE, WILLIAM, Sc.D., LL.D., New York Zoological Park, New York 60, N.Y. 1946.

BENNETT, JAMES PERCY, Ph.D., Professor Emeritus of Plant Physiology, 151 Hilgard Hall, *University of California*, Berkeley, Calif. 1943.

BESSEY, ERNST A., Ph.D., Professor Emeritus of Botany, *Michigan State College*, East Lansing, Mich. 1931.

BIGELOW, HENRY B., Ph.D., Museum of Comparative Zoology, *Harvard University*, Cambridge, Mass. 1910.

BORING, ALICE M., Ph.D., Professor Emeritus of Biology, *Yenching University*, China, 44 Martin St., Cambridge 38, Mass. 1911.

BRADLEY, JAMES C., Ph.D., Professor Emeritus of Entomology, *Cornell University*, Ithaca, N.Y. 1910.

BROWNE, WILLIAM WARD, Ph.D., Dept. of Bacteriology, *College of City of New York*, 139th St. and Convent Ave., New York, N.Y. 1914.

BRUES, CHARLES THOMAS, M.S., A.M., Professor Emeritus of Entomology, Biological Laboratories, *Harvard University*, Cambridge, Mass. 1913.

BUCHANAN, R. E., Ph.D., Emeritus Dean of Graduate College, *Iowa State College*, 503 Welch Ave., Ames, Iowa. 1932.

CALVERT, PHILIP P., Ph.D., Professor Emeritus of Zoology, *University of Pennsylvania*, P. O. Box 14, Cheyney, Pa. 1913.

CARLSON, ANTON J., Ph.D., M.D., D.Sc., Professor Emeritus of Physiology, *University of Chicago*, Chicago, Ill. 1931.

CAROTHERS, E. ELEANOR, Ph.D., 134 Ave. C, East, Kingman, Kan. 1916.

CHAMBERLIN, RALPH V., Ph.D., Sc.D., Professor Emeritus of Zoology, *University of Utah*, Salt Lake City, Utah. 1914.

CHAMBERS, ROBERT, Ph.D., LL.D., Dept. of Biology, *Washington Square College*, New York University, 425 Riverside Drive, New York, N.Y. 1910.

CHANDLER, W. H., Ph.D., Professor Emeritus of Horticulture, *University of California*, 341 South Almont Drive, Beverly Hills, Calif. 1927.

COE, WESLEY R., Ph.D., Professor Emeritus of Biology, *Yale University*, 183 Third Ave., Chula Vista, Calif. 1893.

COKER, ROBERT E., Ph.D., Professor Emeritus of Zoology, *University of North Carolina*, Chapel Hill, N.C. 1929.

COLLINS, J. L., Ph.D., Retired Geneticist, *Pineapple Research Institute of Hawaii*, R. D. 1, Box 369, Alpine, Calif. 1934.

COLTON, HAROLD SELLERS, Ph.D., Professor Emeritus of Zoology, *University of Pennsylvania*, Box 601, Flagstaff, Ariz. 1910.

CONARD, HENRY S., Ph.D., Professor Emeritus of Botany, *Grinnell College*, Grinnell, Iowa, Lake Hamilton, Fla. 1932.

COONS, G. H., Ph.D., Div. of Sugar Plant Investigations, BPI Station, Beltsville, Md. 1937.

CONN, HAROLD J., Ph.D., *New York State Agricultural Experiment Station*, Geneva, N.Y. 1934.

COOPER, WILLIAM S., Ph.D., Sc.D., Professor Emeritus of Botany, *University of Minnesota*, 1421 Bluebell Ave., Boulder, Colo. 1936.

CORT, W. W., Ph.D., School of Public Health, *University of North Carolina*, Chapel Hill, N.C. 1924.

COWDRY, E. V., Ph.D., Director Wernse Cancer Research Lab., *Washington University School of Medicine*, St. Louis 10, Mo. 1925.

CRAMPTON, HENRY E., Ph.D., Sc.D., Professor Emeritus of Zoology, *Columbia University*, American Museum of Natural History, New York, N.Y. 1896.

CURTIS, MAYNIE ROSE, Ph.D., Cancer Research Lab., *University of Miami*, Coral Gables 46, Fla. 1914.

CURTIS, WINTERTON C., Ph.D., Professor Emeritus of Zoology, *University of Missouri*, 210 Westmount Ave., Columbia, Mo. 1904.

DANFORTH, CHARLES H., Ph.D., Sc.D., Professor Emeritus of Anatomy, *Stanford University*, Calif. 1930.

DENNY, F. E., Ph.D., Plant Physiologist Retired, *Boyce Thompson Institute*; 1013 Locust St., N. E. St. Petersburg, Fla. 1932.

DODGE, BERNARD O., Ph.D., *New York Botanical Garden*, New York, N.Y. 1928.

DORSEY, MAXWELL J., Ph.D., Dept. of Horticulture, *University of Illinois*, 1502, South Lincoln Ave., Urbana, Ill. 1931.

EAMES, ARTHUR J., Ph.D., Professor Emeritus of Botany, *New York State College of Agriculture, Cornell University*, Ithaca, N.Y. 1922.

EDWARDS, DAYTON J., Ph.D., Professor Emeritus of Physiology, *Cornell Medical College*, 1300 York Ave., New York, N.Y. 1914.

EHLERS, JOHN H., Ph.D., Biologic Station, *University of Michigan*, Ann Arbor, Mich. 1936.

EVANS, ALEXANDER W., Ph.D., Professor Emeritus of Botany, *Yale University*, 180 Livingston St., New Haven, Conn. 1893.

EYSTER, WILLIAM H., Ph.D., 235 Harrison St., Emmaus, Pa. 1932.

FAULL, JOSEPH H., Ph.D., Professor Emeritus of Forest Pathology, Arnold Arboretum, 72 Fresh Pond Lane, Cambridge 38, Mass. 1929.

FIELD, WILLIAM L. W., A.M., Head Master Emeritus, *Milton Academy*, Milton, Mass. 1910.

GARBER, RALPH J., Ph.D., U.S. Regional Pasture Research Lab., U.S.D.A., State College, Pa. 1928.

GARNER, W. W., Ph.D., Bureau of Plant Industry, U.S.D.A., 1367 Parkwood Place N. W., Washington 10, D.C. 1922.

GATES, WILLIAM H., Sc.D., Professor Emeritus of Zoology, *Louisiana State University*, R. D. 3, Perkins Road, Baton Rouge, La. 1932.

GEROULD, JOHN H., Ph.D., Professor Emeritus of Zoology, *Dartmouth College*, 36 Occom Ridge, Hanover, N.H. 1904.

GILBERT, EDWARD M., Ph.D., Professor Emeritus of Botany, *University of Wisconsin*, Madison, Wis. 1928.

GOLDFORB, A. J., Ph.D., Professor Emeritus of Biology, *College of the City of New York*, New York 31, N.Y. 1910.

GOODALE, H. D., Ph.D., Biologist, Mt. Hope Farm, 257 W. Main St., Williamstown, Mass. 1921.

GREGORY, WILLIAM K., Ph.D., D.Sc., Professor Emeritus of Vertebrate Paleontology, *Columbia University*, Woodstock, N.Y. 1917.

GUDGER, EUGENE W., Ph.D., Honorary Associate Curator of Ichthyology, *American Museum of Natural History*, Waynesville, N.C. 1925.

GULICK, ADDISON, Ph.D., Department of Physiological Chemistry, *University of Missouri*, 308 Westmount Ave., Columbia, Mo. 1946.

GUYER, MICHAEL FREDERIC, Ph.D., Dept. of Zoology, *University of Wisconsin*, Madison, Wis. 1904.

HADLEY, PHILIP B., Ph.D., Research Bacteriologist Retired, *Western Pennsylvania Hospital*, Pittsburgh; Camp Beavertail, Cedarville, Mich. 1922.

HAHN, CLARENCE W., M.S., 3314 Murray Lane, Flushing, N.Y. 1910.

HANKINS, FRANK H., Ph.D., Professor Emeritus of Sociology, *Smith College*, 197 Elm St., Northampton, Mass. 1936.

HARGITT, GEORGE T., Ph.D., Sc.D., Emeritus Professor of Zoology, *Duke University*, Durham, N.C. 1932.

HARMAN, MARY T., Ph.D., Professor Emeritus of Zoology, *Kansas State College*, Manhattan, Kan. 1928.

HART, GEORGE H., V.M.D., M.D., Emeritus Dean, *University of California School of Veterinary Medicine*, Davis, Calif. 1934.

HARTMAN, CARL G., Ph.D., Physiology and Pharmacology Div., Ortho Research Foundation, Raritan, N.J. 1924.

HAYES, HERBERT K., Sc.D., Professor Emeritus of Agronomy and Plant Genetics, University Farm, *University of Minnesota*, St. Paul, Minn. 1911.

HERRICK, CHARLES J., Ph.D., Sc.D., Professor Emeritus of Neurology, *University of Chicago*; 236 Morningside Drive, Grand Rapids, Mich. 1904.

HEUSER, CHESTER, Ph.D., Dept. of Microscopic Anatomy, *Medical College of Georgia*, Augusta, Ga. 1915.

HUNT, HARRISON R., Ph.D., Professor Emeritus of Zoology, *Michigan State College*, East Lansing, Mich. 1931.

HUTCHISON, C. B., LL.D., Emeritus Dean, College of Agriculture, *University of California*, Berkeley 4, Calif. 1931.

JACKSON, HARTLEY H. T., Ph.D., Room 61, U.S. National Museum, Washington 25, D.C. 1914.

JACOBS, M. H., Ph.D., Professor Emeritus of General Physiology, Medical Laboratories, *University of Pennsylvania*, Philadelphia, Pa. 1913.

JOHANNSEN, OSKAR A., Ph.D., Professor Emeritus of Entomology, *Cornell University*, 203 Parkway, Ithaca, N.Y. 1923.

JORDAN, HARVEY E., Ph.D., Sc.D., Retired Dean, School of Medicine, *University of Virginia*, Charlottesville, Va. 1911.

KEARNEY, THOMAS H., Ph.D., LL.D., *California Academy of Science*, San Francisco 18, Calif. 1914.

KEPNER, WILLIAM A., Ph.D., Sc.D., Professor Emeritus of Biology, *University of Virginia*, 29 University Place, Charlottesville, Va. 1931.

KIESSELBACH, T. A., Ph.D., Professor Emeritus of Agronomy, *University of Nebraska*, Lincoln, Neb. 1932.

KILLIP, ELLSWORTH P., A.B., Dept. of Botany, U.S. National Museum, Smithsonian Inst., Washington 25, D.C. 1946.

KNUDSEN, LEWIS, Ph.D., Professor Emeritus of Botany, *Cornell University*, Ithaca, N.Y. 1929.

KRAUS, E. J., Ph.D., Dept. of Botany, *Oregon State College*, Corvallis, Ore. 1927.

KUDO, RICHARD R., D.Sc., Institute of Microbiology, *Rutgers University*, New Brunswick, N.J. 1946.

KUNKEL, L. O., Ph.D., Rockefeller Inst. for Medical Research, 66th St. and York Ave., New York 31, N.Y. 1931.

LA RUE, GEORGE R., Ph.D., Professor Emeritus of Zoology, *University of Michigan*, 7203 Wells Parkway, Hyattsville, Md. 1931.

LEWIS, IVEY F., Ph.D., Professor Emeritus of Biology, *University of Virginia*, 1110 Rugby Road, Charlottesville, Va. 1921.

LINK, GEORGE K., Ph.D., Professor Emeritus of Plant Pathology, Dept. of Botany, *University of Chicago*, Chicago, Ill. 1931.

LOVE, HARRY H., Ph.D., Agricultural Exp. Station, *Cornell University*, Ithaca, N.Y. 1911.

MacDOWELL, EDWIN C., Sc.D., Carnegie Inst. of Washington, Cold Spring Harbor, N.Y. 1916.

MACHT, DAVID I., M.D., Phar.D., Litt.D., F.A.C.P., Sinai Hospital Lab., Baltimore 5, Md. 1929.

MATHESON, ROBERT, Ph.D., Dept. of Entomology, *Cornell University*, Ithaca, N.Y. 1935.

MAVOR, JAMES W., Ph.D., 8 Gracewood Park, Cambridge 38, Mass. 1927.

McATEE, WALDO LEE, A.M., 3 Davis Circle, Chapel Hill, N.C. 1931.

McCLURE, CHARLES F. W., Sc.D., Professor Emeritus of Comparative Anatomy, *Princeton University*, Princeton, N.J. 1891.

McINDOO, NORMAN E., Ph.D., 7225 Blair Road, N. W., Washington 12, D.C. 1942.

MEEK, WALTER J., Ph.D., Emeritus Professor of Physiology, *University of Wisconsin*, Madison, Wis. 1932.

MEINECKE, E. P. MICHAEL, Ph.D., 3157 Jackson St., San Francisco, Calif. 1935.

MELANDER, AXEL L., Sc.D., 4670 Ladera Lane, Riverside, Calif. 1934

MELHUS, IRVING E., Ph.D., Dept. of Botany, *Iowa State College*, 407 Pearson Ave., Ames, Iowa. 1931.

MERRILL, ELMER D., Sc.D., LL.D., Professor Emeritus of Botany, Arnold Arboretum, Jamaica Plains, Boston, Mass. 1930.

MOORE, BARRINGTON, M. F., 340 West 23rd St., New York, N.Y. 1929.

MOORE, GEORGE T., Ph.D., Director Emeritus, *Missouri Botanical Garden*, St. Louis, Mo. 1911.

MOORE, J. PERCY, Ph.D., Professor Emeritus of Zoology, *University of Pennsylvania*, Highland Ave., Route 2, Media, Pa. 1913.

MORGAN, ANN H., Ph.D., Dept. of Zoology, *Mount Holyoke College*, South Hadley, Mass. 1919.

MURNEEK, ANDREW E., Ph.D., Dept. of Horticulture, *University of Missouri*, Columbia, Mo. 1936.

NABOURS, ROBERT K., Ph.D., Dept. of Zoology, *Kansas State College*, Manhattan, Kan. 1917.

NEEDHAM, JAMES G., M.S., Litt.D., Ph.D., Sc.D., Professor Emeritus of Entomology, *Cornell University*, 6 Needham Place, Ithaca, N.Y. 1904.

NEWMAN, HORATIO HACKETT, Ph.D., D.Sc., 173 Devon Drive, Clearwater, Fla. 1918.

NICE, LEONARD B., Ph.D., Professor Emeritus of Physiology, *Chicago Medical School*, Chicago 12, Ill. 1915.

OSTERHOUT, WINTHROP J. V., Ph.D., Member Emeritus of Rockefeller Institute for Medical Research, 66th St. and York Ave., New York 21, N.Y. 1916.

PACKARD, CHARLES, Ph.D., *Marine Biological Laboratory*, Woods Hole, Mass. 1932.

PEARSE, ARTHUR S., Ph.D., Professor Emeritus of Zoology, *Duke University*, Durham, N.C. 1914.

PEEBLES, FLORENCE, Ph.D., 380 Rosemont Ave., Pasadena 3, Calif. 1902.

PETRUNKEWITCH, ALEXANDER, Ph.D., D.Sc., Professor Emeritus of Zoology, *Osborn Zoological Laboratory*, New Haven, Conn. 1910.

POOL, RAYMOND J., Ph.D., Professor Emeritus of Botany, *University of Nebraska*, Lincoln 8, Neb. 1931.

RAND, FREDERICK V., Ph.D., 10608 Nash Place, Kensington, Md. 1943.

RAND, HERBERT W., Ph.D., Associate Professor Emeritus of Zoology, *Harvard University*, 7 Siders Pond Road, Falmouth, Mass. 1914.

REED, GEORGE M., Ph.D., Curator of Plant Pathology Retired, *Brooklyn Botanical Garden*; 25 South Linwood Ave., Pittsburgh 5, Pa. 1937.

RICE, EDWARD L., Ph.D., Sc.D., Professor Emeritus of Zoology, *Ohio Wesleyan University*; 791 Weeks St., Palo Alto, Calif. 1896.

RICHARDS, AUTE, Ph.D., Professor Emeritus of Zoology, *University of Oklahoma*; 2950 East Mabel St., Tucson, Ariz. 1913.

RIDDLE, OSCAR, Ph.D., LL.D., Route 4, Plant City, Fla. 1915.

ROBERTS, ELMER, Ph.D., Professor Emeritus of Animal Genetics, Dept. of Animal Science, *University of Illinois*, Urbana, Ill. 1931.

ROSENDAHL, CARL OTTO, Ph.D., Professor Emeritus of Botany, *University of Minnesota*, Minneapolis, Minn. 1934.

RUTHVEN, ALEXANDER G., Ph.D., LL.D., Sc.D., *University of Michigan*; Ann Arbor, Mich. 1916.

SATINA, SOPHIE A., Dept. of Botany, *Smith College*, Northampton, Mass. 1933.

SCHRAMM, JACOB R., Ph.D., Professor Emeritus of Botany, *University of Pennsylvania*, Philadelphia, Pa. 1919.

SCOTT, GEORGE G., Ph.D., Professor Emeritus of Biology, *College of the City of New York*; 460 Henkel Circle, Winter Park, Fla. 1910.

SCOTT, JOHN W., Ph.D., Professor Emeritus of Zoology, *University of Wyoming*; 1409 Garfield St., Laramie, Wyo. 1913.

SHAFER, GEORGE D., Ph.D., Professor Emeritus of Physiology, *Stanford University*; 321 Melville Ave., Palo Alto, Calif. 1911.

SHARP, LESTER W., Ph.D., Sc.D., Professor Emeritus of Botany, *Cornell University*; Neuvo, Calif. 1922.

SHEAR, CORNELIUS L., Ph.D., 1707 North Sixth St., Monroe, La. 1922.

SHELFORD, VICTOR E., Ph.D., *University of Illinois*, Vivarium, Wright and Healey Sts., Champaign, Ill. 1918.

SHERFF, EARL E., Ph.D., 1203 South Church St., Hastings, Mich. 1946.

SHULL, CHARLES A., Ph.D., Professor Emeritus of Plant Physiology, *University of Chicago*; 42 Oakwood Road, Asheville, N.C. 1923.

SMITH, GILBERT M., Ph.D., D.Sc., Professor Emeritus of Botany, *Stanford University*, Palo Alto, Calif. 1928.

SNODGRASS, ROBERT E., A.B., 3706 13th St., N. W., Washington 10, D.C. 1935.

STAKMAN, ELVIN C., Ph.D., Div. of Plant Pathology, *University of Minnesota*, University Farm, St. Paul, Minn. 1921.

STARK, MARY B., Ph.D., Sc.D., Harris, Minn. 1919.

STOUT, ARLOW B., Ph.D., *New York Botanical Garden*, New York 58, N.Y. 1913.

TASHIRO, SHIRO, Ph.D., Dept. of Biochemistry, College of Medicine, *University of Cincinnati*, Cincinnati, Ohio. 1928.

THOM, CHARLES, Ph.D., Port Jefferson, N.Y. 1936.

TILDEN, JOSEPHINE E., M.S., Professor Emeritus of Botany, *University of Minnesota*; Box 37, R. D. 1, Hesperides, Lake Wales, Fla. 1931.

TRANSEAU, EDGAR N., Ph.D., Professor Emeritus of Botany, *Ohio State University*, Columbus, Ohio. 1918.

TURNER, CLARENCE L., Ph.D., Professor Emeritus of Zoology, *Northwestern University*, Evanston, Ill. 1931.

VAN NAME, WILLARD G., Ph.D., *American Museum of Natural History*, 77th and Central Park West, New York, N.Y. 1899.

WAITE, FREDERICK C., Ph.D., Professor Emeritus of Embryology, *Western Reserve University*; 144 Locust St., Dover, N.H. 1904.

WELCH, PAUL S., Ph.D., Professor Emeritus of Zoology, *University of Michigan*, Ann Arbor, Mich. 1934.

WELLS, BERTRAM W., Ph.D., Professor Emeritus of Botany, *State College*, Raleigh, N.C. 1942.

WENRICH, DAVID H., Ph.D., Professor Emeritus of Zoology, *University of Pennsylvania*, Philadelphia, Pa. 1916.

WENTWORTH, EDWARD N., M.S., Red Oak Ridge, R. D. I, Chesterton, Ind. 1911.

WHITE, ORLAND E., Sc.D., 1708 Jefferson Park Ave., Charlottesville, Va. 1914.

WHITING, PHINEAS W., Ph.D., Professor Emeritus of Zoology, *University of Pennsylvania*, Philadelphia, Pa. 1913.

WHITNEY, DAVID D., Ph.D., Professor Emeritus of Zoology, *University of Nebraska*, Lincoln, Neb. 1911.

WIEMAN, HARRY L., Ph.D., Professor Emeritus of Zoology, *University of Cincinnati*, Cincinnati, Ohio. 1913.

WRIGHT, ALBERT H., Ph.D., Professor Emeritus of Zoology, *Cornell University*, Ithaca, N.Y. 1925.

ACTIVE MEMBERS

ADAMS, AMY ELIZABETH, Ph.D., Dept. of Zoology, *Mt. Holyoke College*, South Hadley, Mass. 1939.

ADAMS, MARK H., Ph.D., 43 Woolsey St., Huntington, N.Y. 1953.

ADOLPH, EDWARD F., Ph.D., School of Medicine, Dept. of Physiology, *University of Rochester*, Rochester 7, N.Y. 1933.

AIKMAN, JOHN M., Ph.D., Dept. of Botany, *Iowa State College*, Ames, Iowa. 1946.

ALBRECHT, WILLIAM A., Ph.D., College of Agriculture, *University of Missouri*, Columbia, Mo. 1946.

ALEXANDER, MARY L., Ph.D., M.D. Anderson Cancer Hospital and Tumor Institute, Texas Medical Center, Houston 25, Tex. 1955.

ALLARD, R. W., Ph.D., Department of Agronomy, *University of California*, Davis, Calif. 1954.

ALTENBURG, EDGAR, Ph.D., Dept. of Biology, *Rice Institute*, Houston, Tex. 1931.

AMADON, DEAN, Ph.D., Dept. of Birds, *American Museum of Natural History*, Central Park West at 79th St., New York, N.Y. 1953.

ANDERSON, BERTIL G., Ph.D., Dept. of Zoology, *Penn State University*, State College, Pa. 1942.

ANDERSON, NORMAN G., Ph.D., Biology Division, Oak Ridge National Lab., Oak Ridge, Tenn. 1953.

ANDERSON, THOMAS F., Ph.D., Johnson Foundation, *University of Pennsylvania*, Philadelphia 4, Pa. 1951.

ARMSTRONG, PHILIP B., M.D., Dept. of Anatomy, College of Medicine, *Syracuse University*, Syracuse, N.Y. 1942.

ARONSON, LESTER R., Dept. of Animal Behavior, *American Museum of Natural History*, Central Park West at 79th St., New York, N.Y. 1948.

ARTSCHWAEGER, ERNST, Ph.D., Bureau of Plant Industry, U.S.D.A., Box 35, Mesilla Park, New Mexico. 1946.

ATWOOD, K.C., Ph.D., Biology Division, Oak Ridge National Lab., Oak Ridge, Tenn. 1952.

AUSTIN, MARY L., Ph.D., Dept. of Zoology, *Wellesley College*, Wellesley, Mass. 1950.

AVERY, GEORGE S., Jr., Ph.D., *Brooklyn Botanic Garden*, 1000 Washington Ave., Brooklyn 25, N.Y. 1937.

AXELROD, DANIEL I., Ph.D., Dept. of Geology, *University of California*, Los Angeles 24, Calif. 1950.

BAKER, HORACE B., Ph.D., Dept. of Zoology, *University of Pennsylvania*, Philadelphia 4, Pa. 1924.

BAKER, WILLIAM K., Ph.D., Dept. of Zoology, *University of Chicago*, Chicago 37, Ill. 1952.

BALDWIN, J. T., JR., Ph.D., *College of William and Mary*, Williamsburg, Va. 1955.

BALL, ERNEST, Ph.D., Dept. of Botany, *North Carolina State College*, Raleigh, N.C. 1955.

BANGHAM, RALPH V., Ph.D., Dept. of Biology, *College of Wooster*, Wooster, Ohio. 1953.

BATES, MARSTON, Ph.D., Dept. of Zoology, *University of Michigan*, Ann Arbor, Mich. 1951.

BEAMS, HAROLD W., Ph.D., Dept. of Zoology, *State University of Iowa*, Iowa City, Iowa. 1939.

BECKER, ELERY R., Sc.D., Dept. of Zoology, *Iowa State College*, Ames, Iowa. 1946.

BENSON, LYMAN, Ph.D., Dept. of Botany, *Pomona College*, Claremont, Calif. 1955.

BERGER, CHARLES A., Ph.D., Dept. of Biology, *Fordham University*, New York 58, N.Y. 1943.

BERGNER, DOROTHY, Ph.D., Army Chemical Center, 3204 North Calvert St., Baltimore 18, Md. 1933.

BERRY, L. JOE, Ph.D., Dept. of Biology, *Bryn Mawr College*, Bryn Mawr, Pa. 1954.

BERTALANFFY, LUDWIG VON, Ph.D., Psychosomatic Research Institute, Mt. Sinai Hospital and Clinic, Los Angeles 48, Calif. 1953.

BILLINGS, W. D., Ph.D., Dept. of Botany, *Duke University*, Durham, N.C. 1955.

BIRDSELL, J. B., Ph.D., Dept. of Anthropology and Sociology, *University of California*, Los Angeles 24, Calif. 1953.

BLAIR, ALBERT P., Ph.D., Dept. of Zoology, *University of Tulsa*, Tulsa 4, Okla. 1952.

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Published in *The American Naturalist*, Vol. XC, 139–142. 1956.

RECENT SYMPOSIUM TITLES AND SPEAKERS

1933—Boston. Semi-Centennial Celebration. *Biology and Society*
W. M. Wheeler, E. A. Hooton, F. H. Hankins

1934—Pittsburgh. *Cytogenetic Evolutionary Processes and Their Bearing on Evolution Theory*
R. A. Brink, M. Demerec, C. L. Fenton

1935—St. Louis. *Early Man in America with Particular Reference to the Southwestern United States*
Edgar B. Howard, Chester Stock, Ernst Antevs, Frank H. H. Roberts, Paul MacClintock, E. H. Sellards

1936—Atlantic City. *Supra-Specific Variation in Nature and in Classification*
Alfred C. Kinsey, Edgar Anderson, George Gaylord Simpson, William K. Gregory

1937—Indianapolis. *The Nature of Protoplasm*
W. M. Stanley, S. C. Brooks, Robert Chambers

1938—Richmond. *Contributions of the Higher Animals to an Understanding of Human Biology*
G. K. Noble, C. C. Little, Carl G. Hartman

1939—Columbus. *Defense Mechanisms in Plants and Animals*
F. W. Went, W. C. Price, William Bloom

1940—Philadelphia. *Experimental Control of Differentiation and Development*
A. F. Blakeslee, G. W. Beadle and E. L. Tatum, K. V. Thimann, B. H. Willier

1941—Dallas. *Human Genetics*
L. H. Snyder, H. H. Strandskov, C. W. Cotterman, Lionel S. Penrose

1944—Cleveland. *Biology and Human Progress*
Charles V. Taylor, Edmund W. Sinnott and Robert Chambers

1946—St. Louis. *Recent Advances in the Dynamics of Behavior*
R. Lorente de Nó, M.D., Hudson Hoagland, Curt P. Richter and Carney Landis

1946—Boston. *Genetic Aspects of Natural Populations*
Th. Dobzhansky, Carl Epling, Jens Clausen

1947—Chicago. *Genetics of Microorganisms*
W. M. Stanley and C. A. Knight, S. E. Luria, M. Demerec, B. McClintock and J. R. Singleton, E. C. Stakman, G. W. Keitt

1948—Washington, D.C. *Immunology in Biological Research*
Felix Haurowitz, William C. Boyd, M. R. Irwin

1949—New York. *Reproduction in Plants*
M. W. Parker and H. A. Borthwick, B. Esther Struckmeyer, F. G. Gustafson, F. W. Went

1950—Columbus. *Golden Jubilee Program: Genetics, the First Fifty Years*
Richard Goldschmidt; Conway Zirkle, W. E. Castle, H. J. Muller; A. H. Sturtevant, L. J. Stadler; Hugo Iltis; L. H. Snyder; A. E. Mirsky, T. Caspersson, Jack Schultz, M. R. Irwin; G. W. Beadle; Boris Ephrussi; Joshua Lederberg, T. M. Sonneborn; Th. Dobzhansky; M. J. D. White, C. D. Darlington; J. W. Gowen, C. C. Little; Arne Müntzing, P. C. Mangelsdorf, J. C. Walker, J. L. Lush; Julian Huxley

1951—Minneapolis. *Patterns of Cellular Organization.*
Thomas F. Anderson, Keith R. Porter, Daniel Mazia, John T. Bonner

1951—Philadelphia. *Use of Statistical Models to Interpret Data on Human Population Genetics*
C. C. Li, J. V. Neel, Bentley Glass, J. N. Spuhler and D. J. Hager, Howard Levene

1952—Ithaca. *Biochemical Evolution*
Harold F. Blum, Urless N. Lanham, Sam Granick, Marcel Florkin.

1953—Boston. *Some Biological Effects of Radiation from Nuclear Detonations*
Alan D. Conger; E. B. Lewis; George H. Mickey; Robert Carter, V. P. Bond, James T. Brennan, and E. P. Cronkite; William L. Russell

1954—Gainesville. *The Origin of the Biota of Middle America*
Aaron J. Sharp; Paul S. Martin; Pierce Brodkorb; Coleman J. Goin

1955—East Lansing. *Modern Approaches to Problems of Differentiation*
Tracy M. Sonneborn; John P. Trinkaus; Armin C. Braun; William P. Jacobs

PRESIDENTIAL ADDRESSES AT THE MORE RECENT MEETINGS OF THE AMERICAN SOCIETY OF NATURALISTS

Meeting	*Date*	*Place*	*President*
51st	Dec. 30, 1933	Boston	B. E. Livingston
	Semi-Centennial Anniversary. *Environments*		
52nd	Dec. 29, 1934	Pittsburgh	A. F. Shull
	Weismann and Haeckel: One Hundred Years		
53rd	Jan. 2, 1936	St. Louis	John C. Merriam
	The Extent and Rate of Human Evolution—The Asking of Critical Questions		
54th	Dec. 31, 1936	Atlantic City	C. E. Allen
	Haploid and Diploid Generations		
55th	Dec. 30, 1937	Indianapolis	D. H. Tennent
	Some Problems in the Study of Photosensitization		
56th	Dec. 30, 1938	Richmond	Robert M. Yerkes
	The Life-History and Personality of the Chimpanzee		
57th	Dec. 30, 1939	Columbus	Ivey F. Lewis
	Cell Reactions		
58th	Jan. 1, 1941	Philadelphia	J. H. Bodine
	The Cell—Some Aspects of Its Functional Ontogeny		
59th	Dec. 31, 1941	Dallas	W. W. Cort
	Human Factors in Parasite Ecology		
60th	Sept. 14, 1944	Cleveland	Fay-Cooper Cole
	Some Problems of Human Racial Development and Migration		
61st	Mar. 30, 1946	St. Louis	Edmund W. Sinnott
	Substance or System, the Riddle of Morphogenesis		
62nd	Dec. 28, 1946	Boston	C. W. Metz
	Duplication of Chromosome Parts as a Factor in Evolution		
63rd	Dec. 29, 1947	Chicago	K. S. Lashley
	Persistent Problems in the Evolution of Mind		
64th	Sept. 11, 1948	Washington, D.C.	P. R. Burkholder
	Studies in the Nutrition of Plants with Pure Culture Techniques		
65th	Dec. 29, 1949		T. M. Sonneborn
	Heredity, Environment, and Politics		
66th	Sept. 13, 1950		Th. Dobzhansky
	Evolutionary Changes in Mendelian Populations		
67th	Sept. 11, 1951		Paul C. Mangelsdorf
	Evolution Under Domestication		
68th	Sept. 9, 1952		Sewall Wright
	Gene and Organism		
69th	Dec. 30, 1953	Boston	L. J. Stadler
	The Gene		
70th	Sept. 7, 1954	Gainesville, Fla.	M. Demerec
	What is a Gene?—Twenty Years Later		
71st	Sept. 7, 1955	East Lansing, Mich.	K. V. Thimann
	Promotion and Inhibition: Twin Themes of Physiology		

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